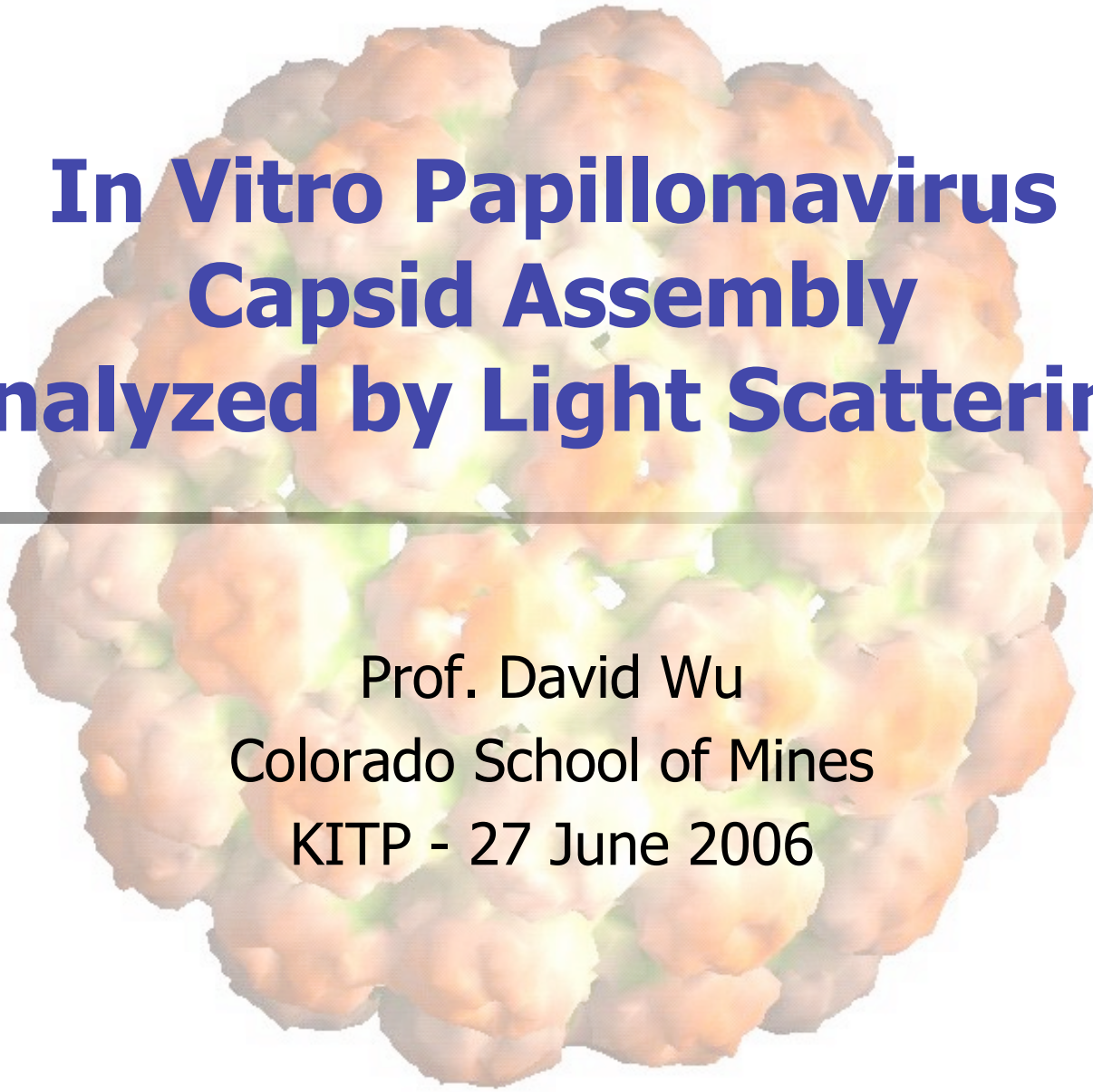
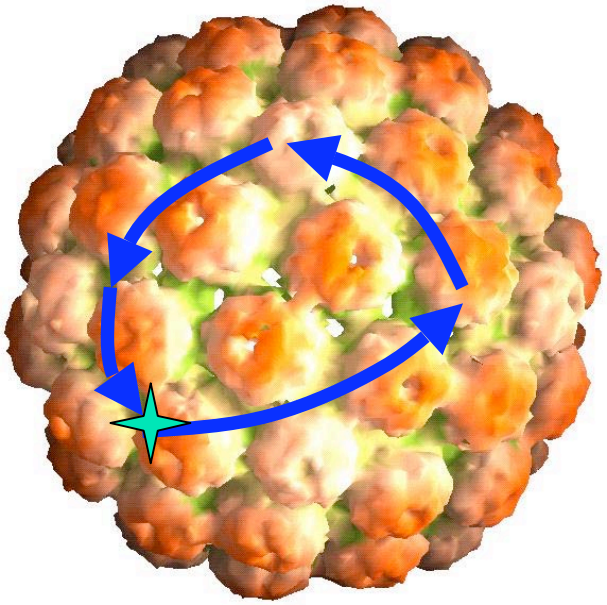


In Vitro Papillomavirus Capsid Assembly Analyzed by Light Scattering

Prof. David Wu
Colorado School of Mines
KITP - 27 June 2006

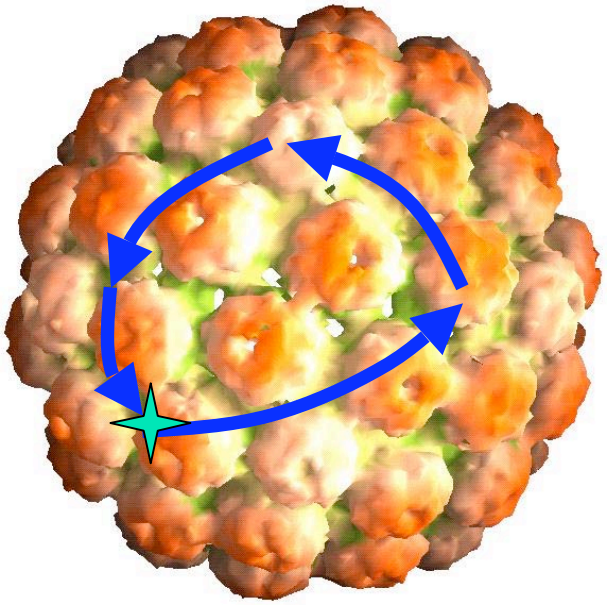
Travelogue

- Origins & trip plans
- Wading into the swamp
- According to Mapquest
- Places to visit next

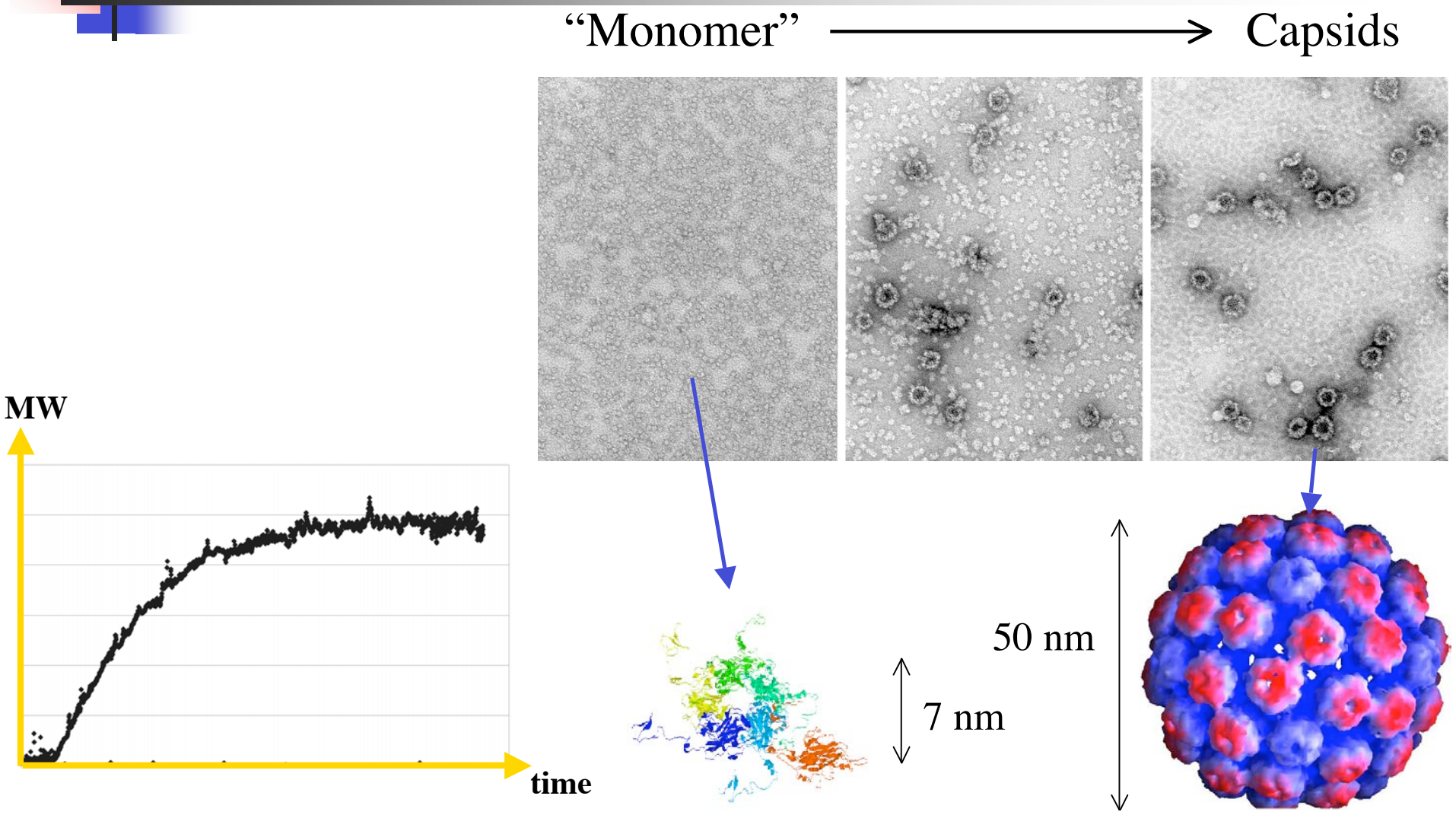


Origins & trip plans

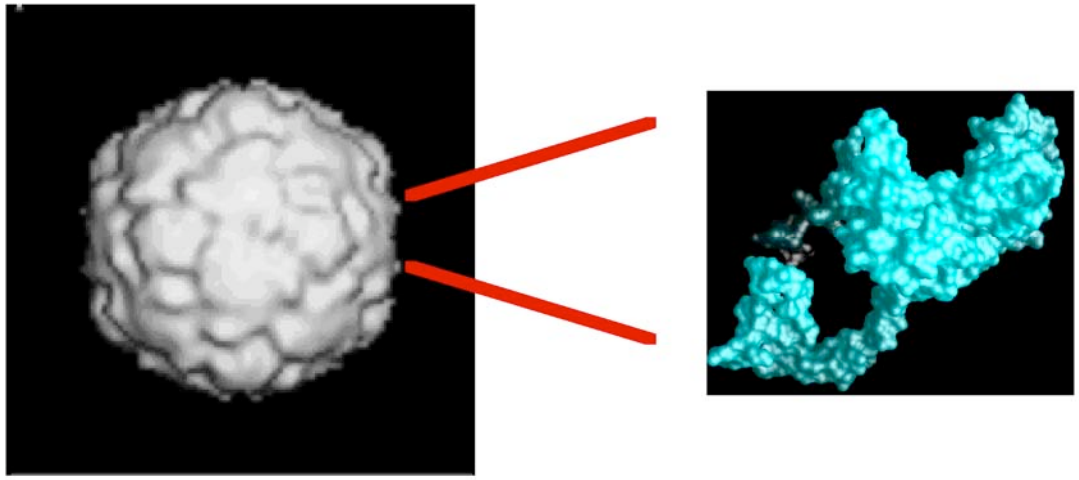
- Where my interest started
- Introduce relevant virology

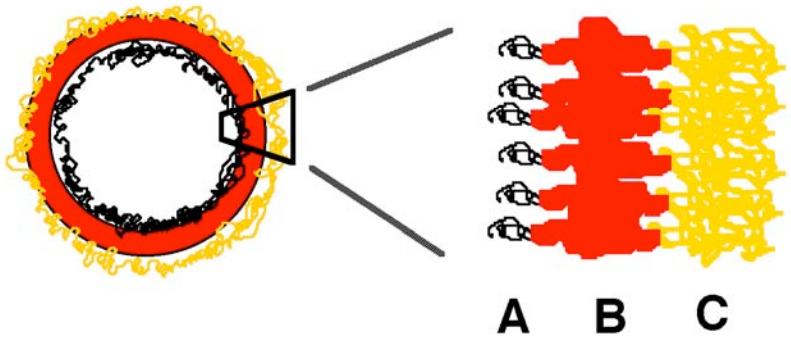


The basic wonder of it is....



A Parallel….?





Essential questions

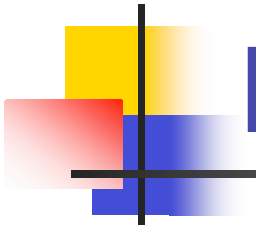
- Questions about the part
  - How much information?
  - Consequences of shape/specific interactions/mechanical properties, etc.
- Questions about the process
  - Mechanisms
  - Cooperativity
  - Thermodynamics & Kinetics
  - Fluctuations

Mechanism

- Is there an identifiable “mechanism” or pathway? (cf. Aggregation)
  - Sequential addition? Subtraction?
- Are there important intermediates?
- Does assembly proceed by “nucleation & growth”?

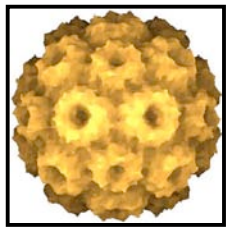
Thermodynamics & kinetics

- Do different structures correspond to thermodynamic or energetic optima?
- Or is structure under kinetic control?
- Competing pathways/products
  - Off-size capsids, off-symmetry capsids, ribbons, winding shells…
- Timescale/rate of assembly
  - Responsive to biological signals



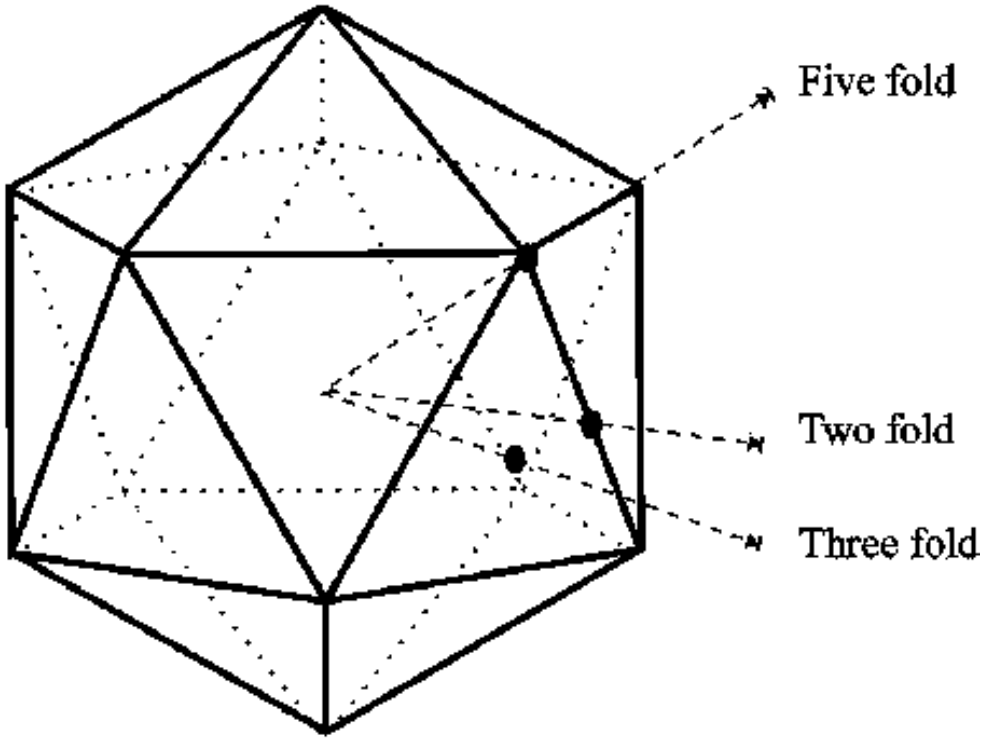
Fluctuations

- Virology has been strong on structure, comparatively weak on dynamics:
  - Is microscopic reversibility relevant?
  - Error correction--does it happen?
  - Functional role for fluctuations?
    - e.g. mechanical/transport
  - Role in protein-protein binding?

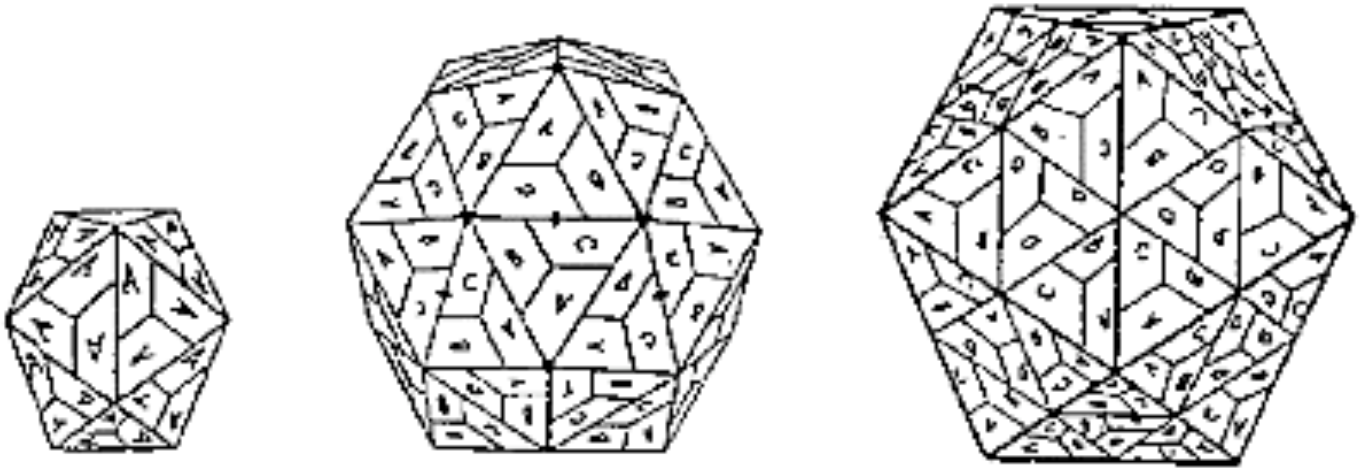


Icosahedral capsids

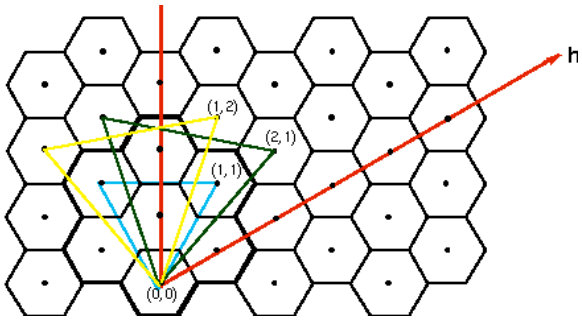
- Icosahedral
  - 20 identical triangles
  - Characterized by a pattern of 5-fold, 3-fold and 2-fold rotational symmetry
  - Large volume/surface area



Quasiequivalence
(Caspar & Klug, 1962)



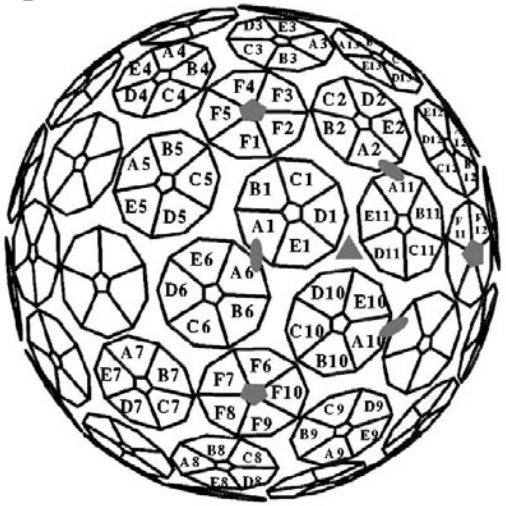
T = 1 T = 3 T = 4

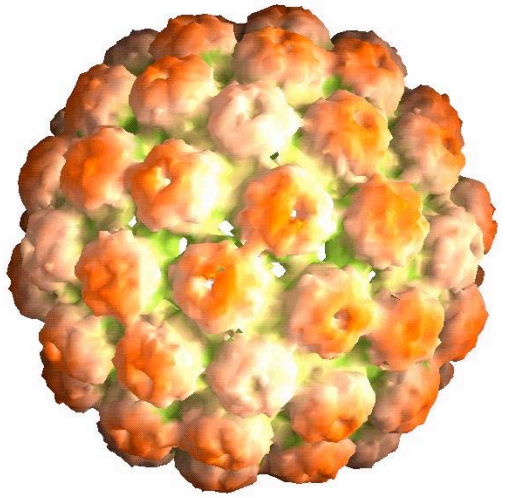


- 60*T copies, with T=1,3,4,7,9,12…
- 12 5-fold and T(10-1) locally 6-fold sites
- Chiral (like fullerenes)

Inequivalent positions

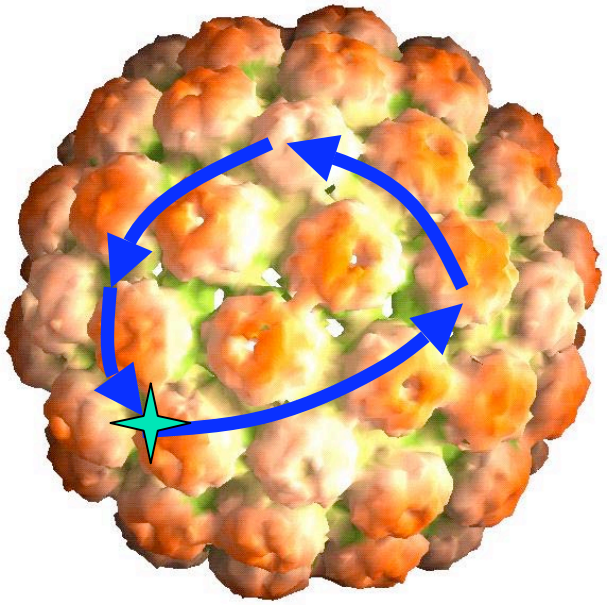
- How do proteins find their correct positions?
- Errors and correction?
- Papilloma, Polyoma, SV40 are exceptions: *not* quasiequivalent
  - T=7d surface lattice
  - Prebonded pentamers
  - "5 around 1" intermediates?



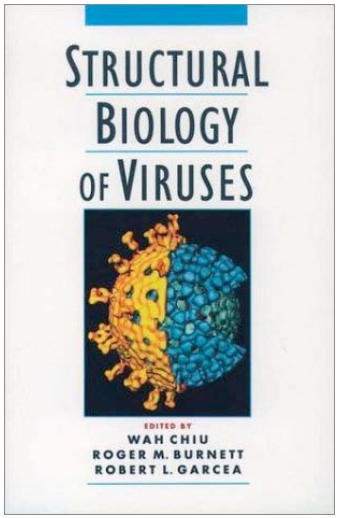


Wandering into the swamp

- MALS light-scattering experiments on capsid assembly
- Track kinetics by average molecular weight

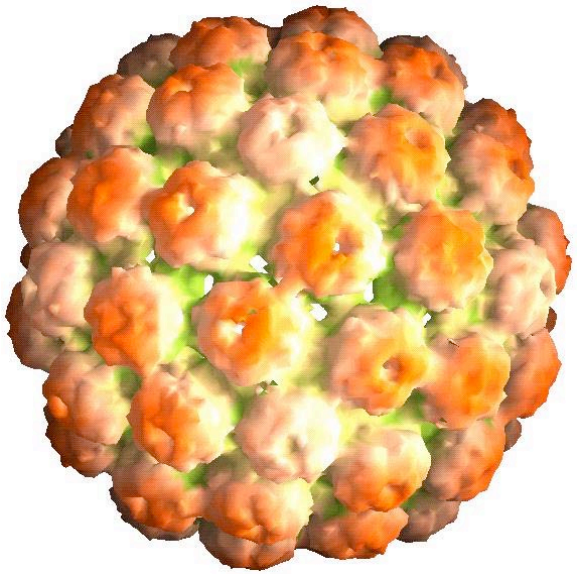


Bob Garcea
UCHSC

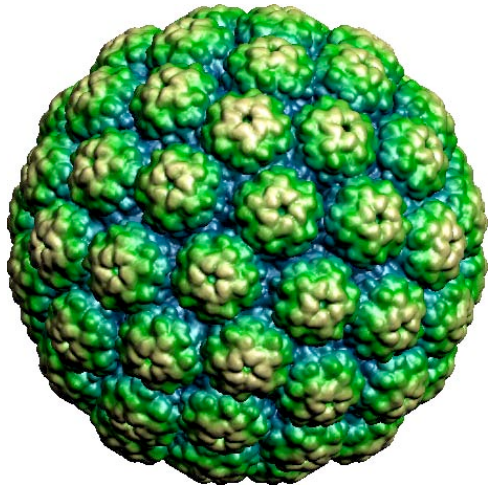


Casini et al., Virology, 2004

Papilloma & Polyoma



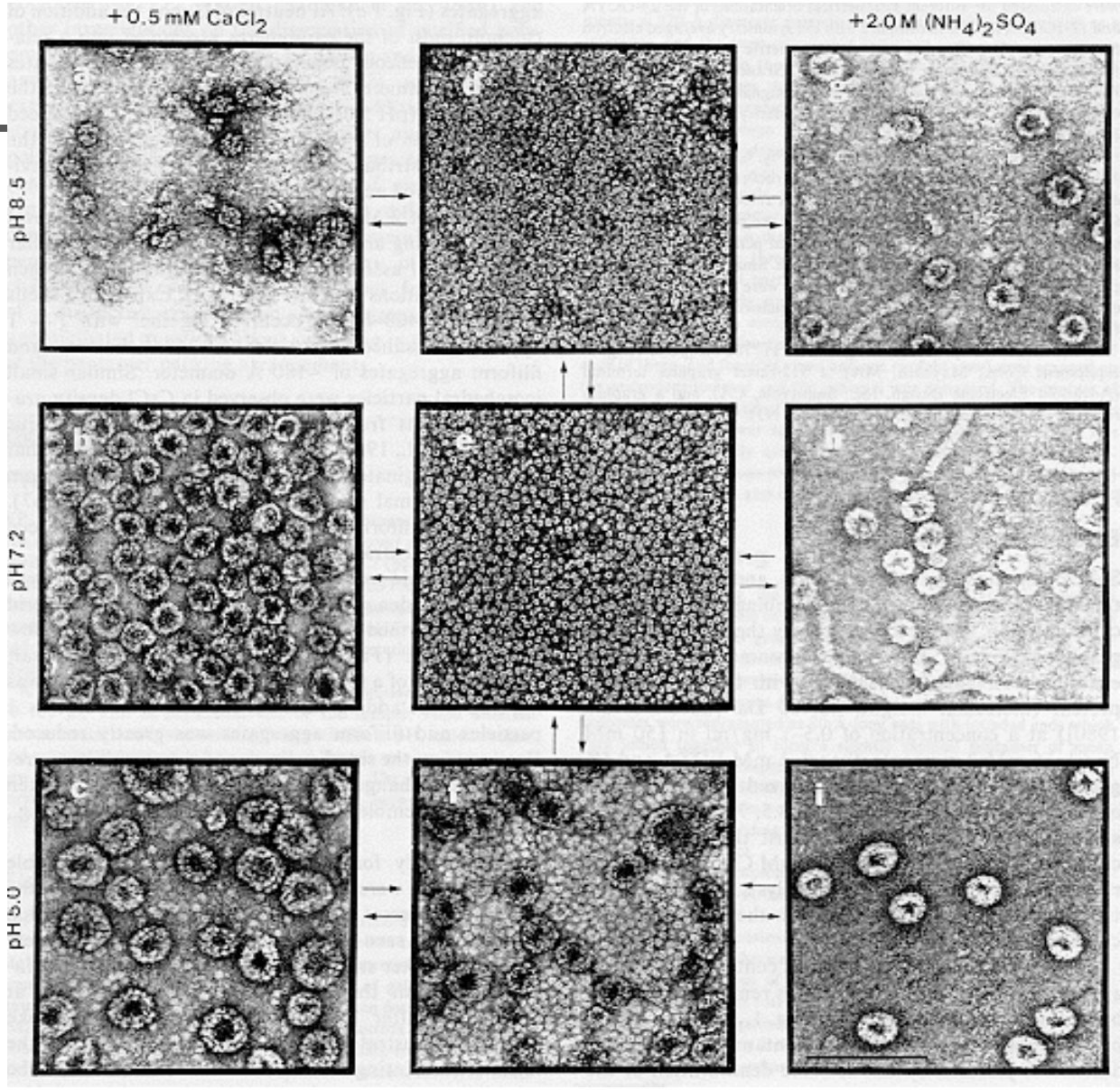
60 nm



50 nm

- Two subfamilies of Papovaviridae
- Structural similarity at tertiary and quaternary (T=7) level, but not secondary

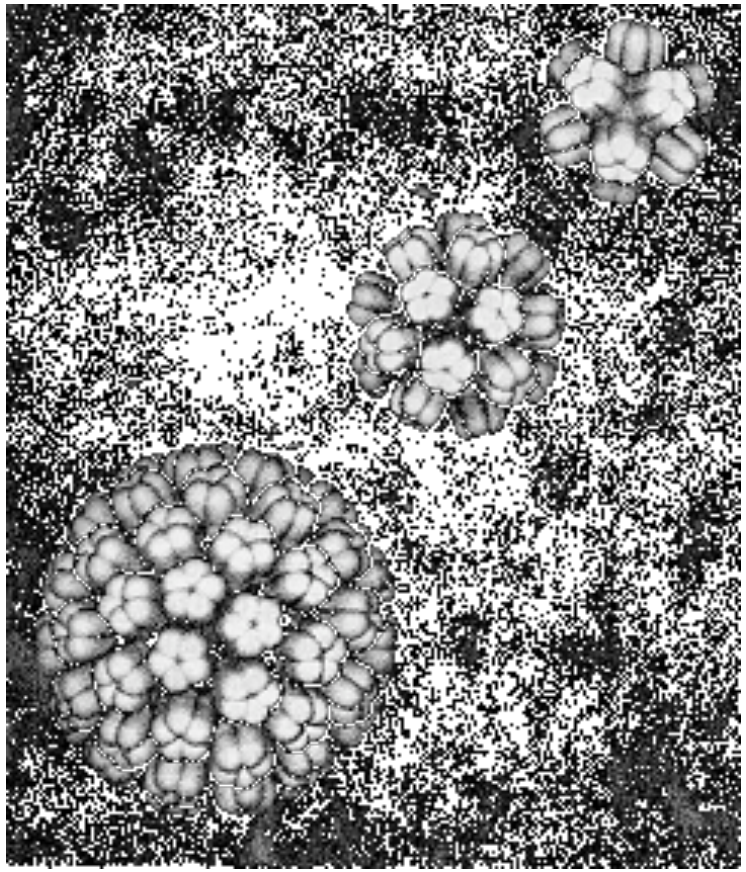
Reversible normal & monster assembly



Polyoma VP1
Garcea et al.
Nature '87

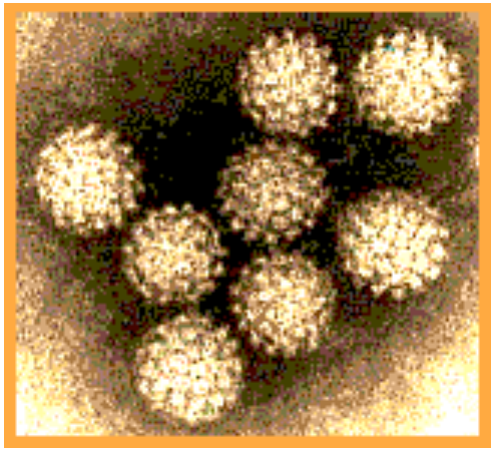
Various symmetries possible

- Three different complete shell assemblies can form:
  - 12-icosa
  - 24-octa
  - 72-icosa
- (12 & 24 are mutant)



Human papillomavirus (HPV)

- >90 strains
- Closed circular 8kb dsDNA in minichromosome
- Two structural proteins
  - Major capsid protein L1 (500 residues)
  - Minor capsid protein L2 (3%)
- Assembly occurs in nucleus of cell, not reducing cytoplasm
- Host chaperones mediate assembly and disassembly



HPV and Cervical Cancer

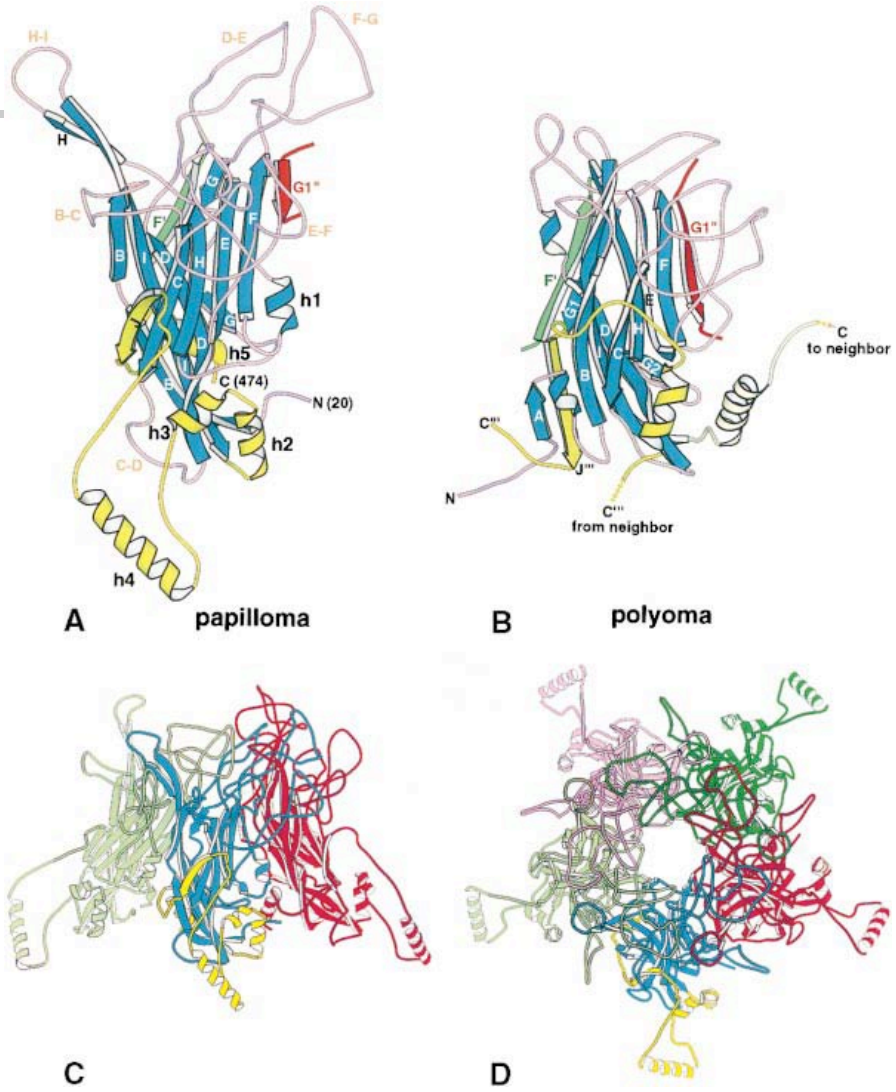
- Cervical Cancer kills 300,000 women/yr worldwide (mostly in developing countries)
  - 2nd after breast cancer, 1st in some developing countries
  - Nearly all cervical cancers show HPV infections
  - 75% of sexually active women have been infected
    - 2×10^7 currently infected worldwide
    - 6×10^6 new HPV infections/yr in US

Capsids for Cancer Vaccine

- HPV Virus-Like Particles (capsids) as vaccine
- Gardasil (Merck) approved June 2006
  - 2nd cancer vaccine
    - ~100% effective in Phase III on 12,000 women in 13 countries
  - Targets HPV 6, 11, 16, 18 VLPs
    - 70% of Cervical cancer cases & 90% genital warts
  - $360/person , expect $2B/yr sales
  - To be administered pre-puberty (two decades from infection to cancer)
  - Gates Foundation $30M grant to study & distribute to poorer countries

Protein and pentamer structure

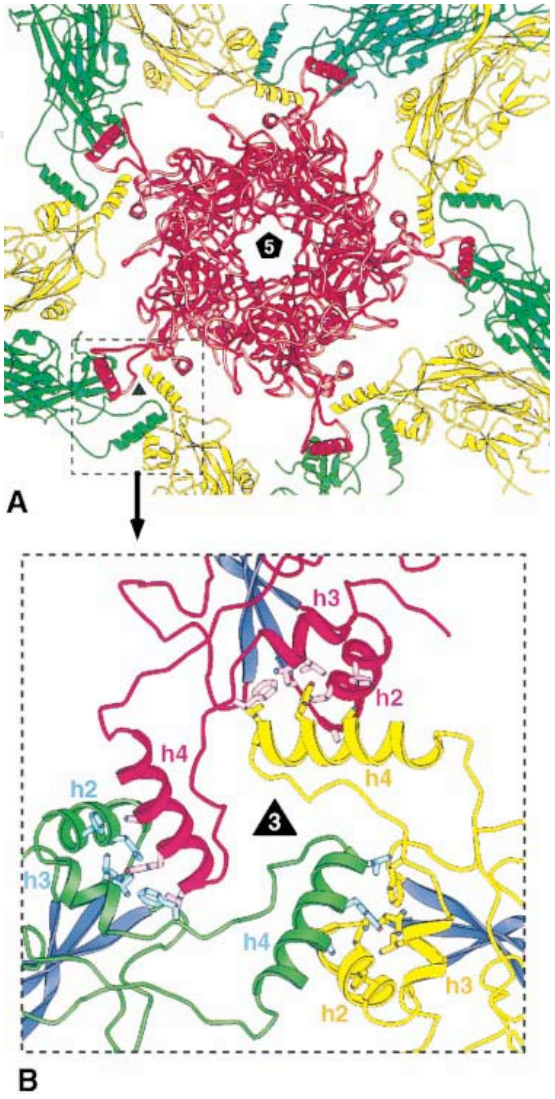
- Classical jelly roll beta sandwich
- HPV
  - Helical subdomain near C-terminus needed for interpentamer contacts for assembly
  - N-terminus controls size (T=7 -> T=1)
  - C-terminus + charged, disordered, inside
- Polyoma
  - N-terminus + charged
  - C-terminus disordered, orders upon invasion of neighboring pentamer, w/ Ca^{2+} contacts



Chen et al., Mol. Cell 2000 ; Chen et al., JMB 2001

T=1 HPV VLP Crystal Structure

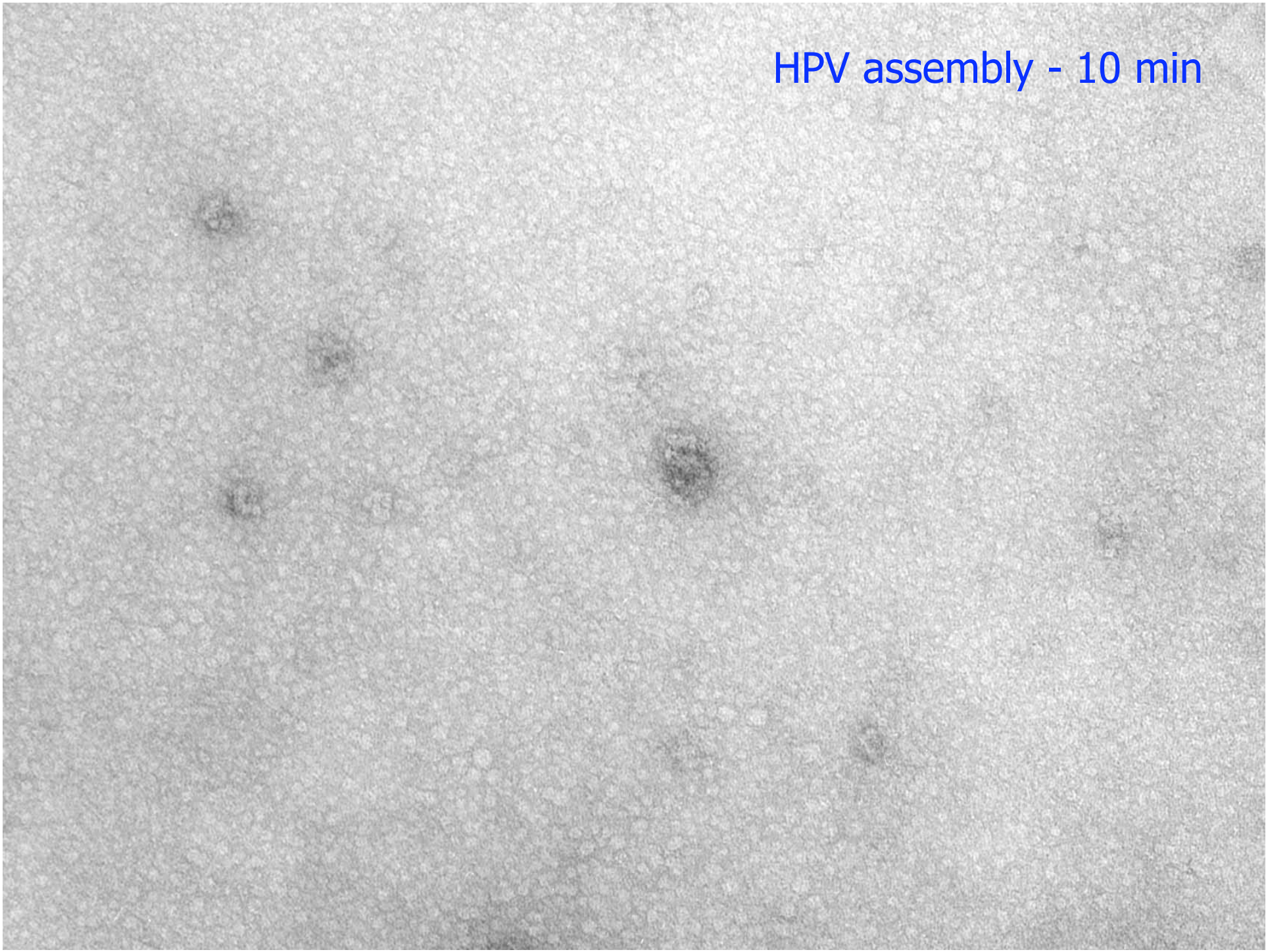
- Helical subdomain mediates 3-fold interpentameric hydrophobic contact
- Two conserved cysteines presumed to form interpentameric disulfide bonds during HVP capsid formation
  - Disulfide bonds in Polyoma are intrapentameric

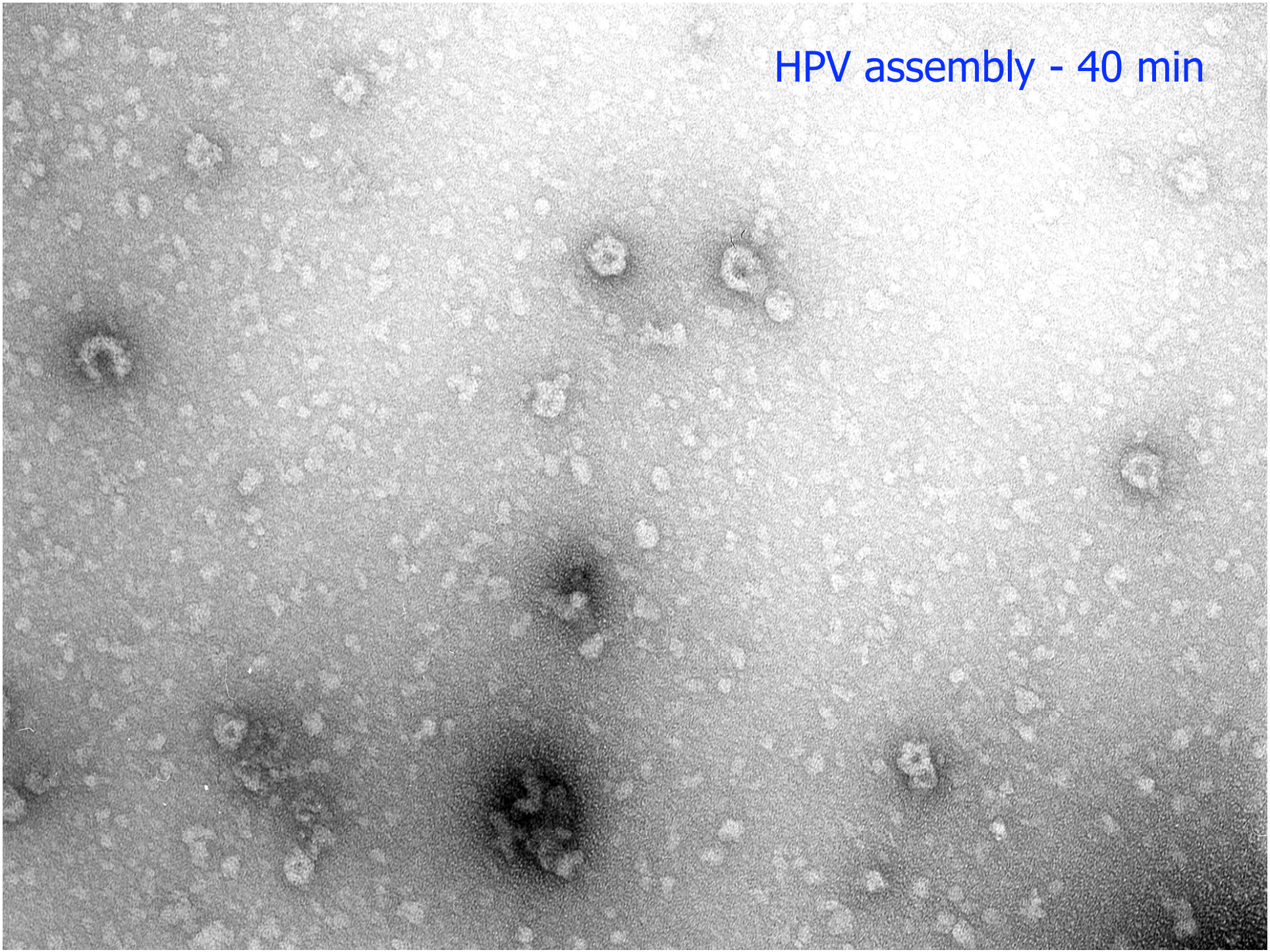


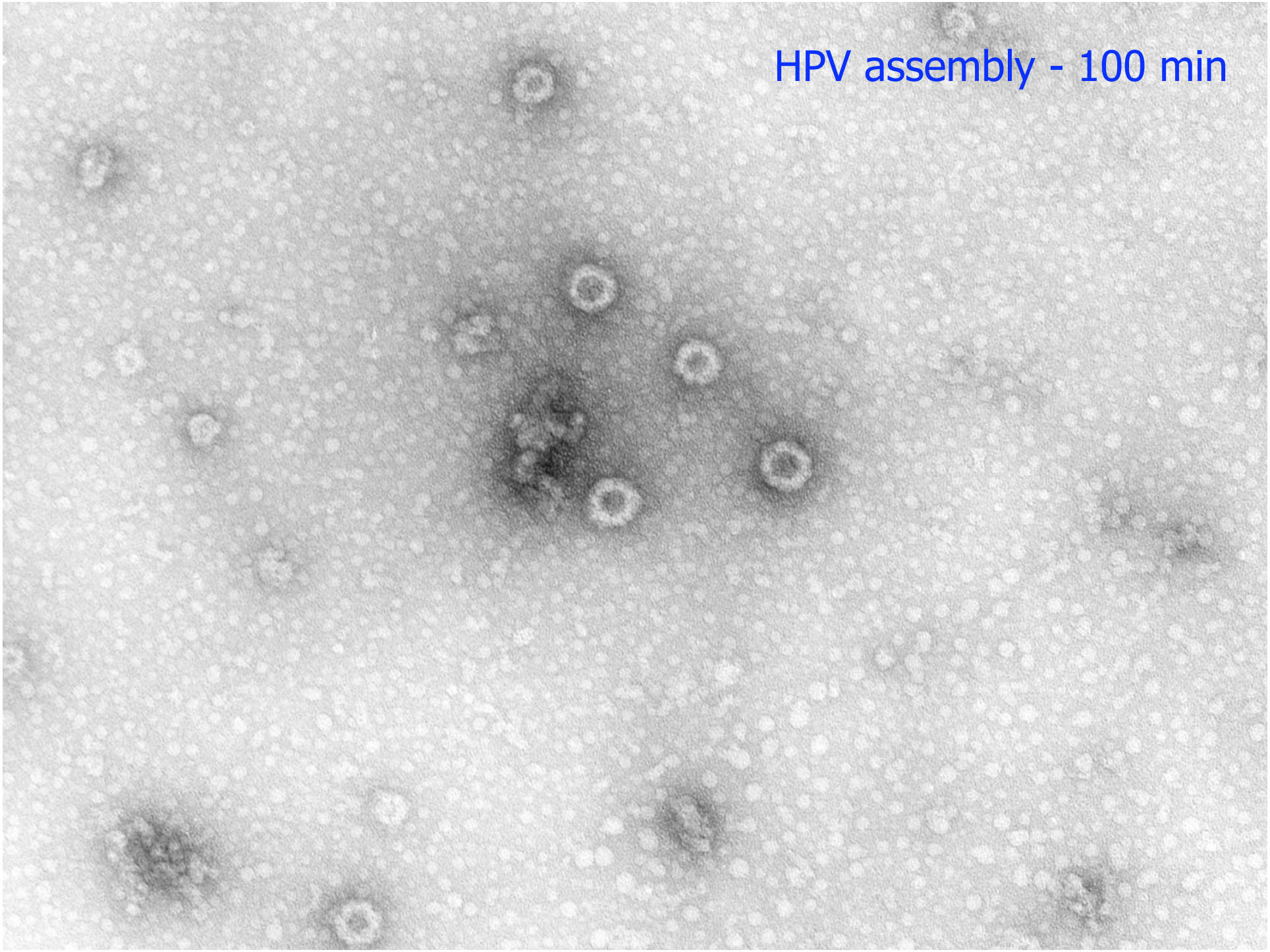
Chen et al., Mol. Cell 2000 ; Chen et al., JMB 2001

HPV Virus Like Particles Structure

- Human Papillomavirus
  - Major Capsid Protein L1 – Type 11
    - Molecular Weight – 55 kD
  - Capsomeres are L1 pentamers – 275 kD
    - 60 Hexavalent Capsomeres
    - 12 Pentavalent Capsomeres
    - Arranged in a T=7 surface lattice
  - Virion Capsid – 20 MD
    - 72 capsomeres = 360 protein units
    - Diameter – 52nm

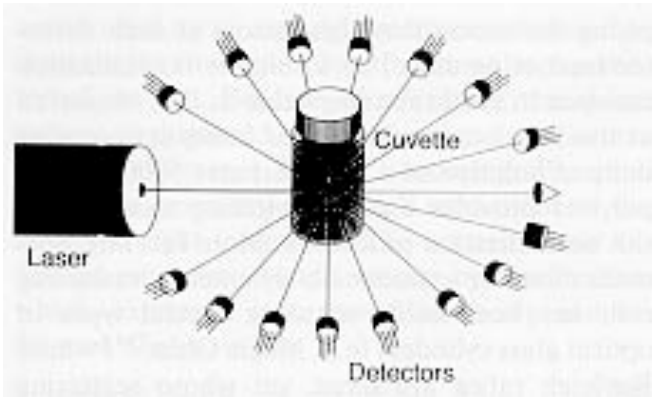

HPV assembly - 10 min


HPV assembly - 40 min


HPV assembly - 100 min

Experimental procedure

- Multi-Angle Light Scattering (MALS)
  - Procedure
  - Absolute MW and R_g
- Data collected
  - Various concentrations
- Analysis
  - Relative rates of assembly
  - Initial growth rate
  - Lag time



Wyatt, P., Analytica Chimica Acta, 272(1993)1-40

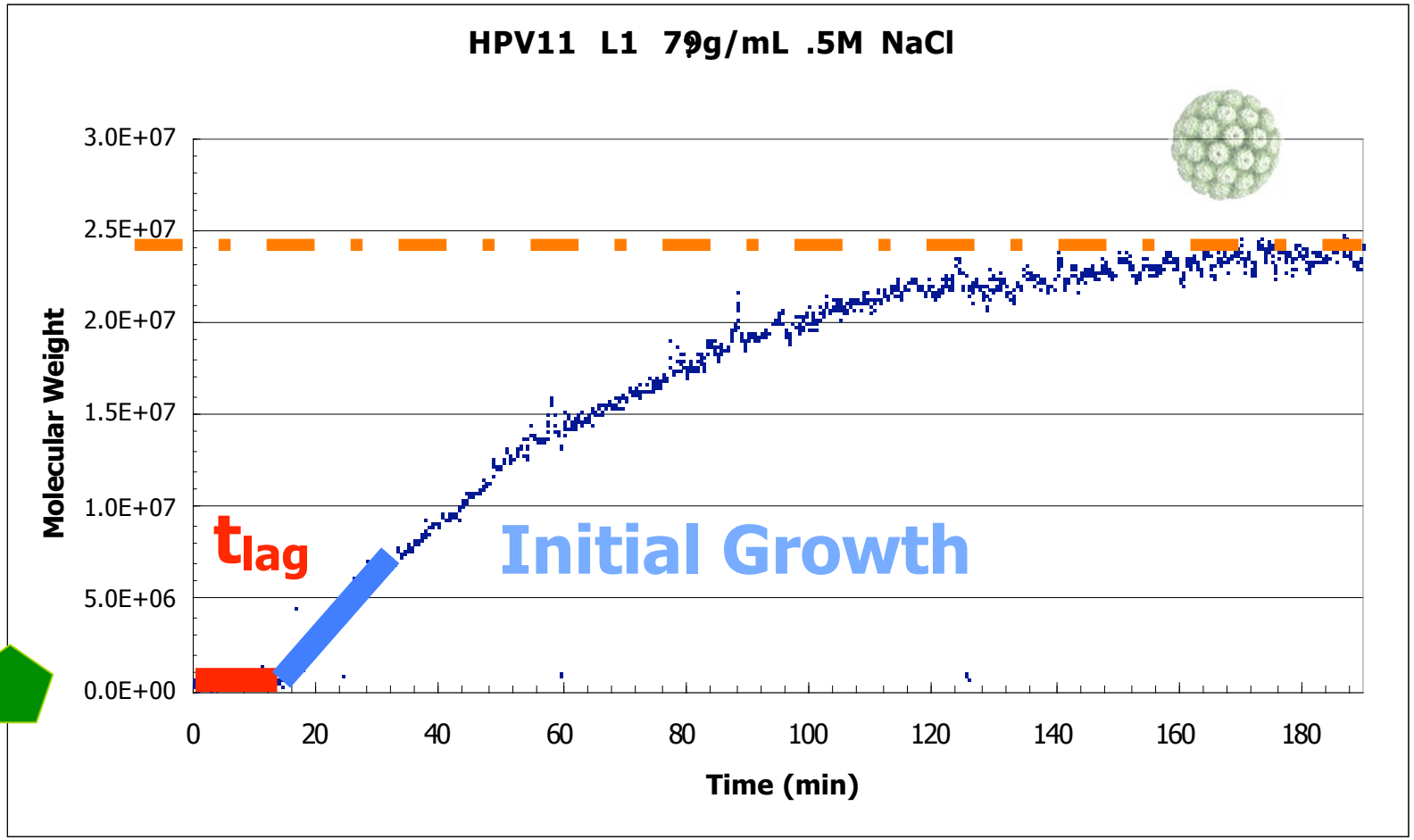
Procedure

- Sample L1 protein is stored in a Tris buffer @ pH 8.0, 0.1M NaCl and a T=-70°C
- Protein introduced to acetate buffer @ pH 5.2 and 0.5M NaCl
- Assembly begins
- Afterwards, the concentration is determined by UV Spectrometry
  - Typical concentration: 20-200μg/mL
- Extrapolation to find molecular weight

$$\frac{R(\Theta)}{K^* c} = M_w \cdot [1 - \frac{16\pi^2 n_o^2 r_g^2}{3\lambda_o^2} \sin^2(\Theta/2)]$$

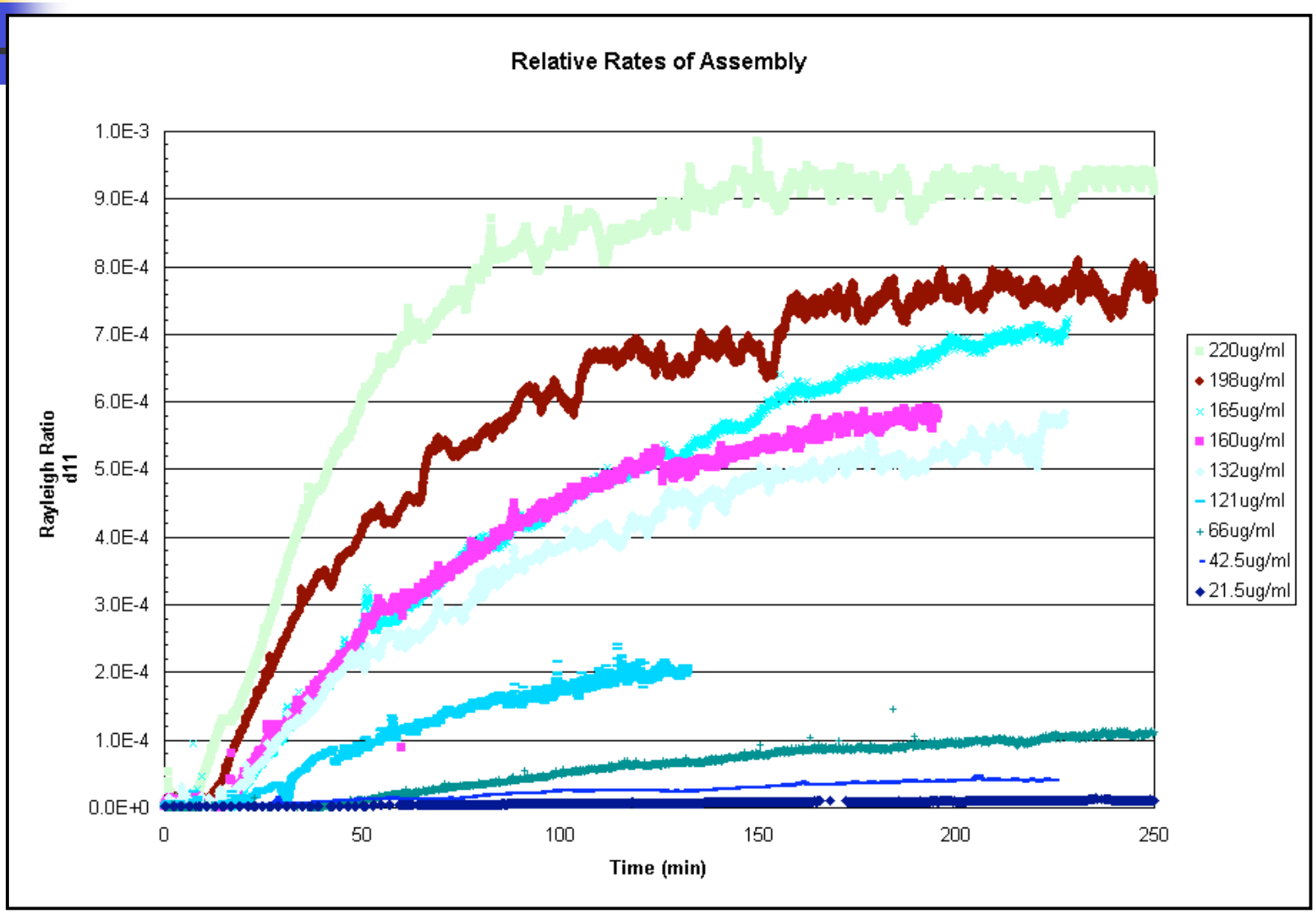
Wyatt, P., Analytica Chimica Acta, 272(1993)1-40

Avg MW growth during assembly



Assembly begun @ t=0

Relative rates of assembly



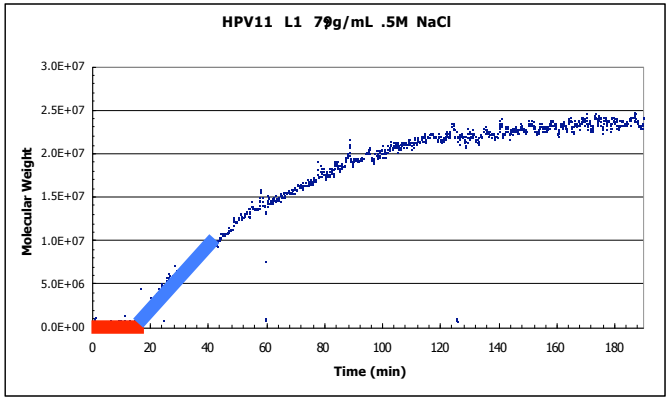
Interpretation of key kinetic features

I. Initial Growth

- What occurs during this period?
- What does the growth relate to?

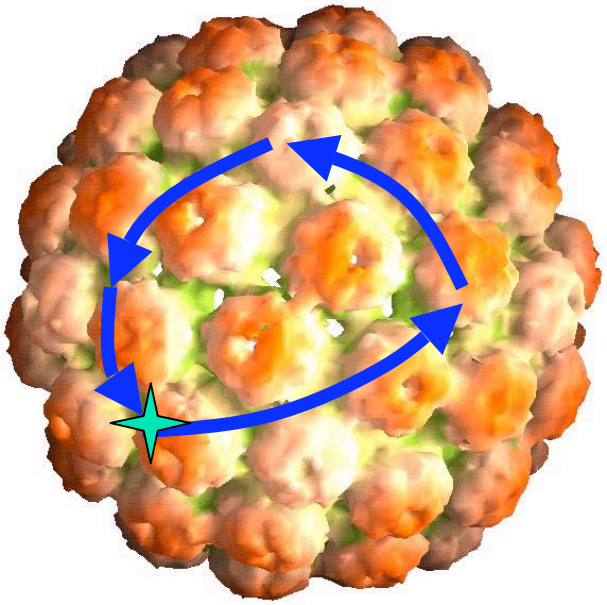
II. Lag Time

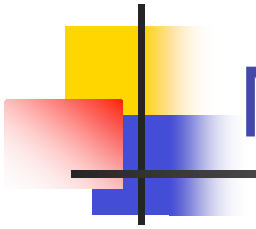
- What is it dependent on?
- Not as sharp in other studies



According to Mapquest…

- Comparison of experiment against sequential growth model (Zlotnick)





Modeling capsid assembly kinetics

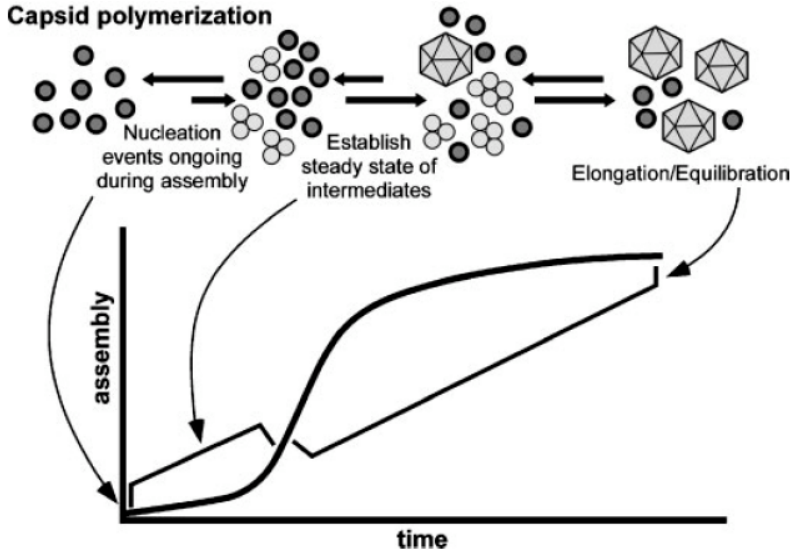
- Tracking large number of reactions and intermediates difficult
- Simulation approaches
  - Kinetic Rate Laws
    - Dominant & Multiple Path Assembly
  - Molecular Simulation

Kinetic rate law simulations

- Simple kinetic simulations have been performed[1]

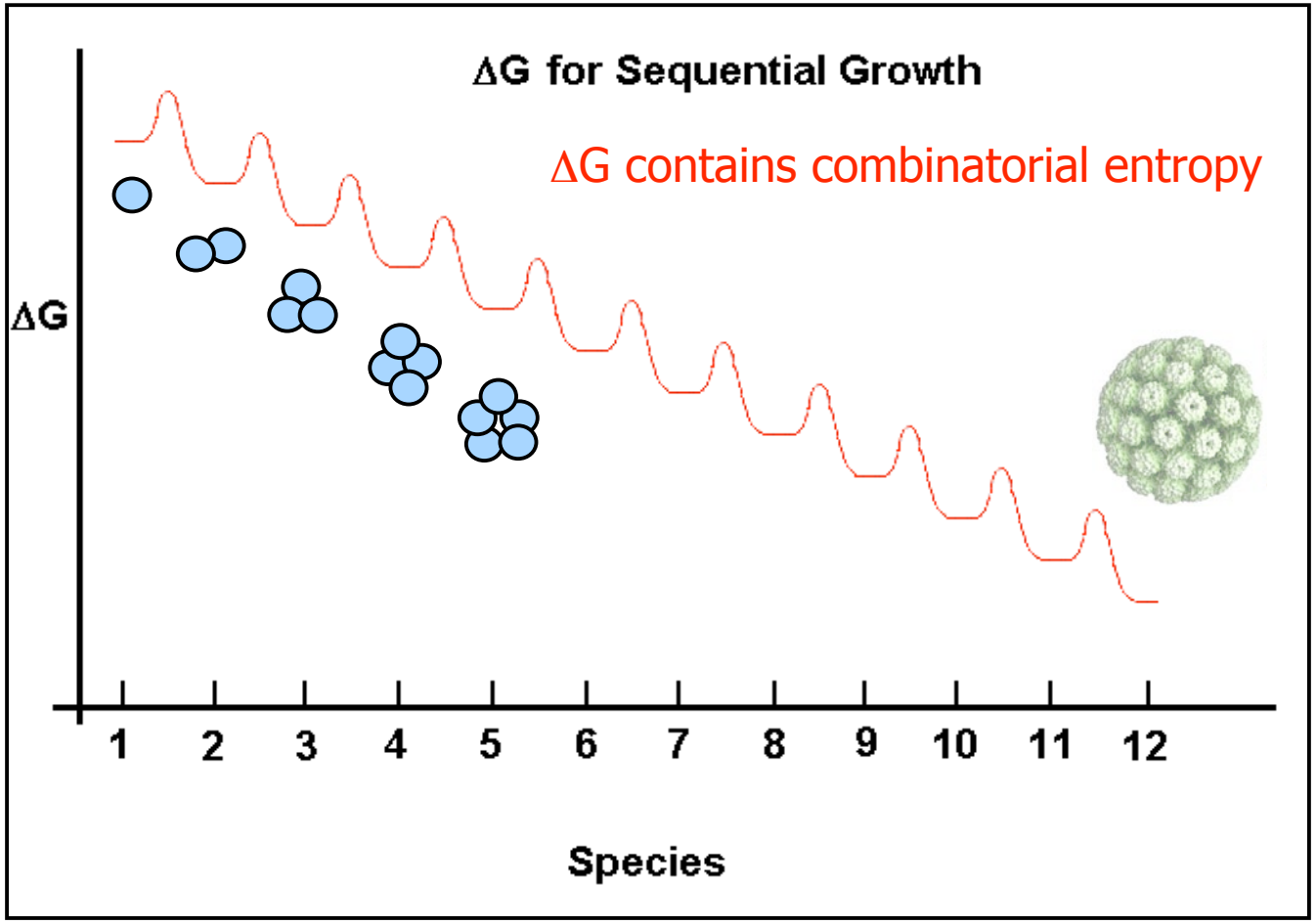
$$\text{subunits} \Leftrightarrow \text{nuclei} + \text{subunits} \Leftrightarrow \text{capsids}$$

- Information obtained
  - Nucleation size
  - Nucleation rate
  - Contact ΔG
  - Elongation rate



1 Zlotnick, J. of Mol. Recognit. **18**, 479-490 2005
2 Zhang, T. et al, Biophys. J. **90** 57-64 2006
3 Schwartz R. et al, Biophys. J. **75** 2626-2636 1998

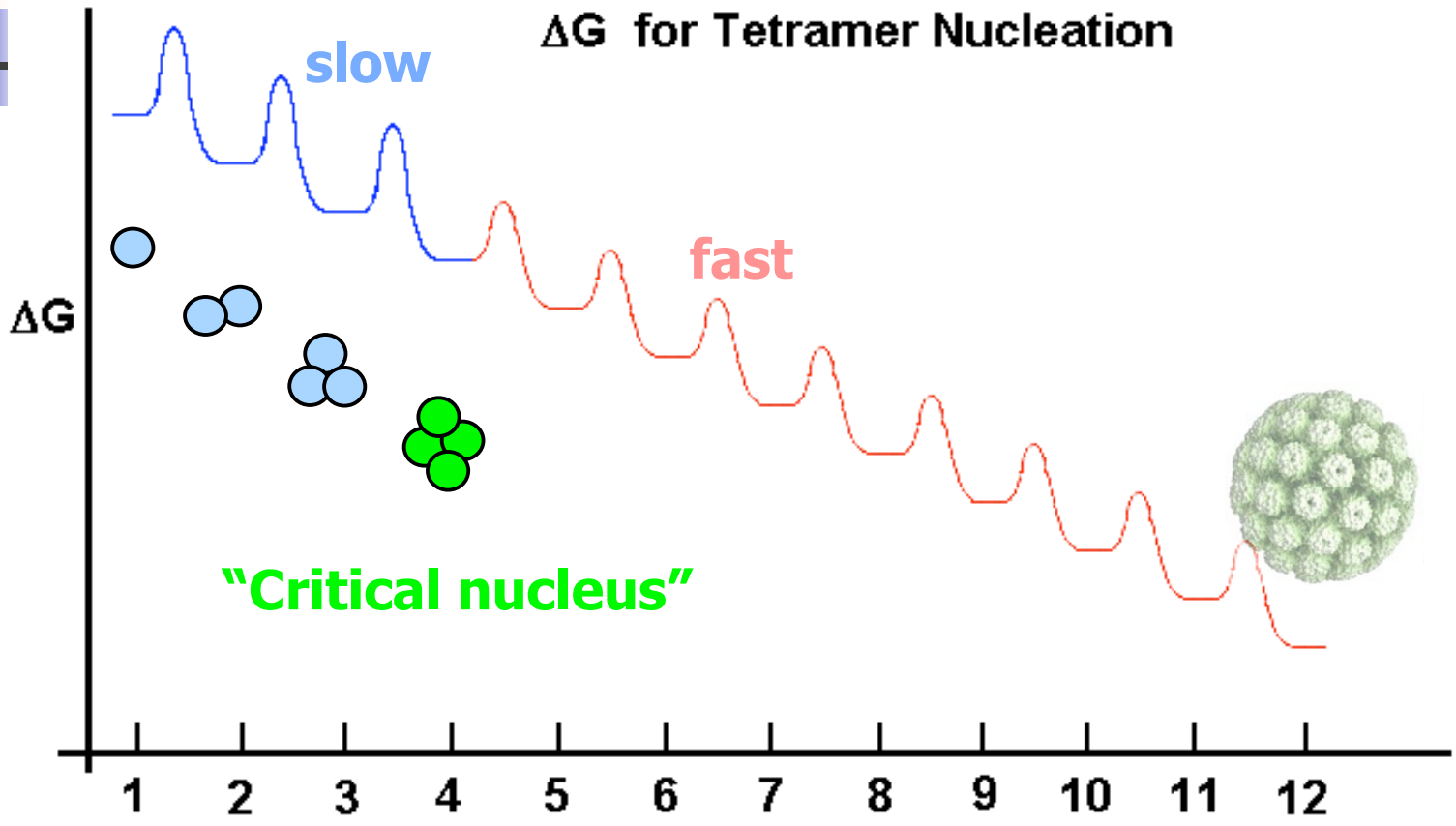
Dominant path "equilibrium" assembly



Zlotnick, A., J.Mol.Biol (1994) 241, 59-67

Flory, Paul, Principles of Polymer Chemistry, 1953

Kinetically limited "nucleation"

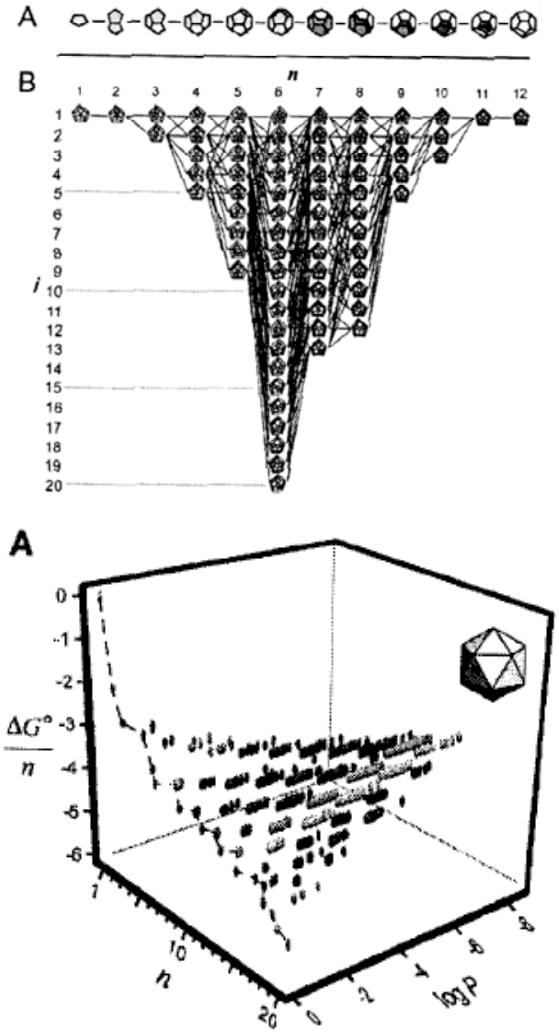


Zlotnick, A. et al., Biochemistry (1999) 28, 14644.

Endres and Zlotnick, Biophys. J. (2002) 1217.

Kinetic rate law simulation: multiple path assembly

- Reaction landscape enumeration[1,2] for 12-mer
  - Access intermediates
  - Limited to simple systems
- Concluded dominant path was sufficiently accurate
- But 12-mer has no quasiequivalence
- Errors neglected



1 Zlotnick, J. of Mol. Recognit. **18**, 479-490 2005
2 Enders, et al., Protein Sci. **14**, 1518-1525 2005

Sequential growth

- Monomer Additions

$$C_1 + C_1 \xleftrightarrow{k_1, k_{-1}} C_2$$

$$C_2 + C_1 \xleftrightarrow{k_2, k_{-2}} C_3$$

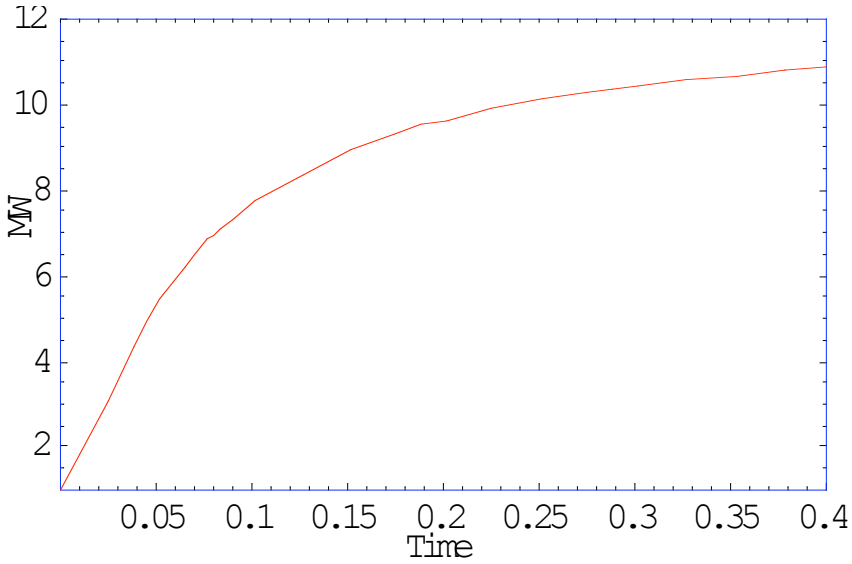
$$C_3 + C_1 \xleftrightarrow{k_3, k_{-3}} C_4 \cdots$$

$$C_{11} + C_1 \xleftrightarrow{k_{11}, k_{-11}} C_{12}$$

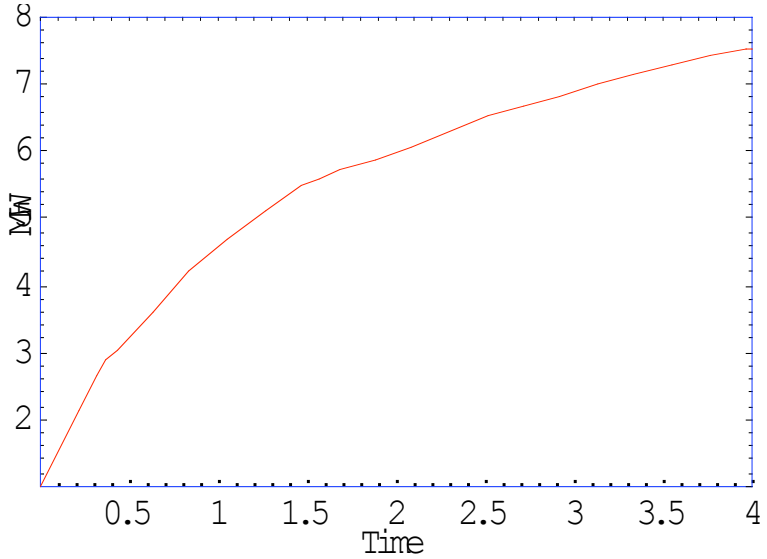
Zlotnick,A., J. Mol. Biol. (1994)241,59-67

Dominant pathway assembly

- Equilibrium
- $k_{forward}=10^8$



- Kinetic Limiting Trimer
- $k_1= k_2 =10^6$
- $k_3= k_4...=10^8$



Lag times tend to be rounded, especially in MW curves

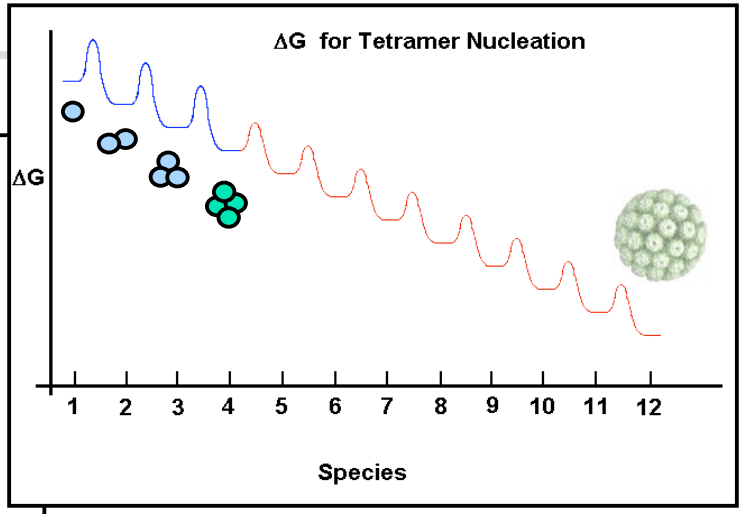
What controls the initial slope?

$$\frac{d[nucleus]}{dt} = k_n[subunit]^N$$

$$[nucleus] \propto [capsid] \propto MW_w$$

$$(slope\ of\ MW_w) = k_n \cdot [C_o]^N$$

$$\log(rate\ of\ LS) = \log(k_n) + N\log[C_o]$$

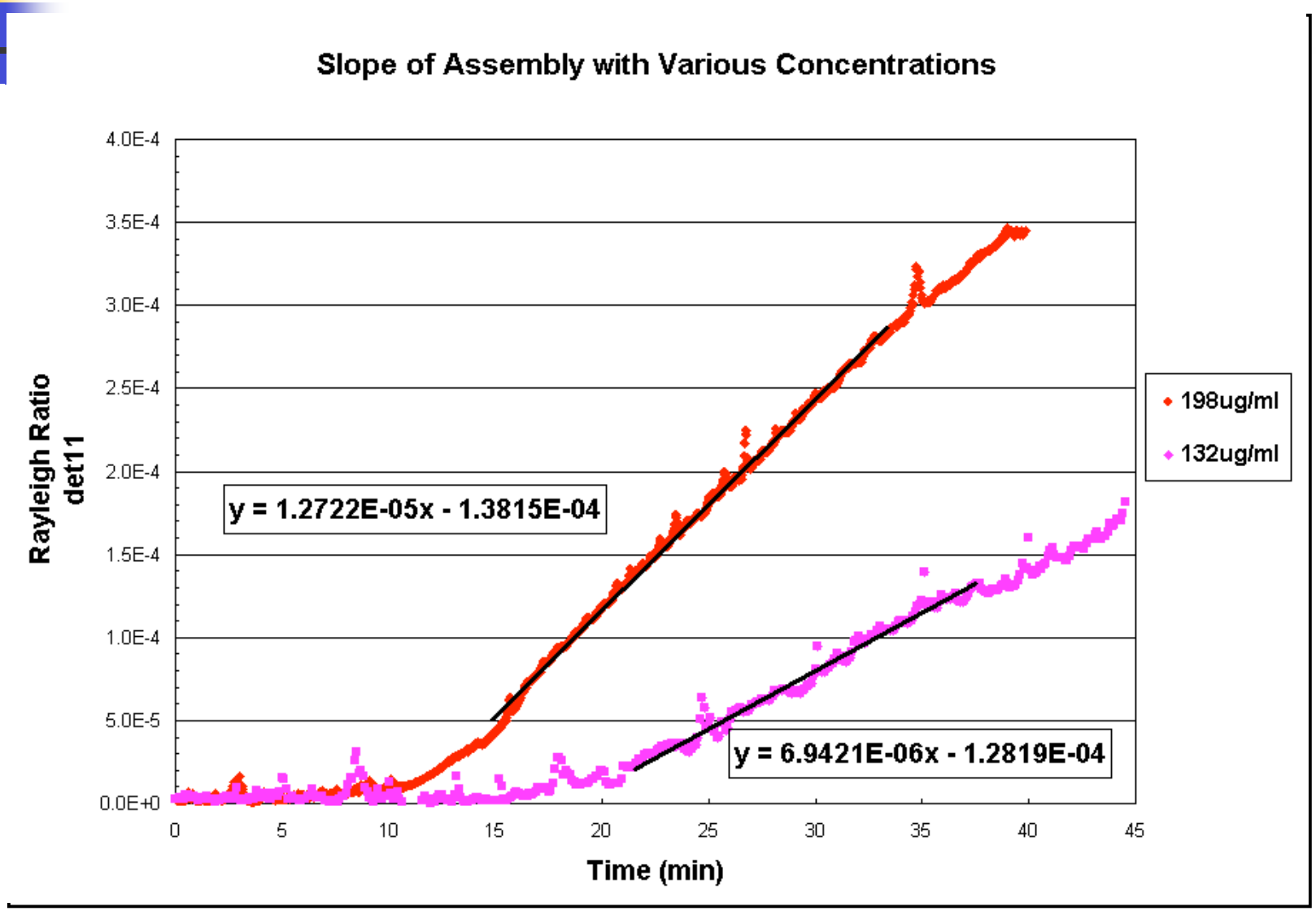


Note: Zlotnick proposed more accurate estimators of the nucleus size based on number of capsids at a given time.
Our curves are quite linear, and so the two give similar results.

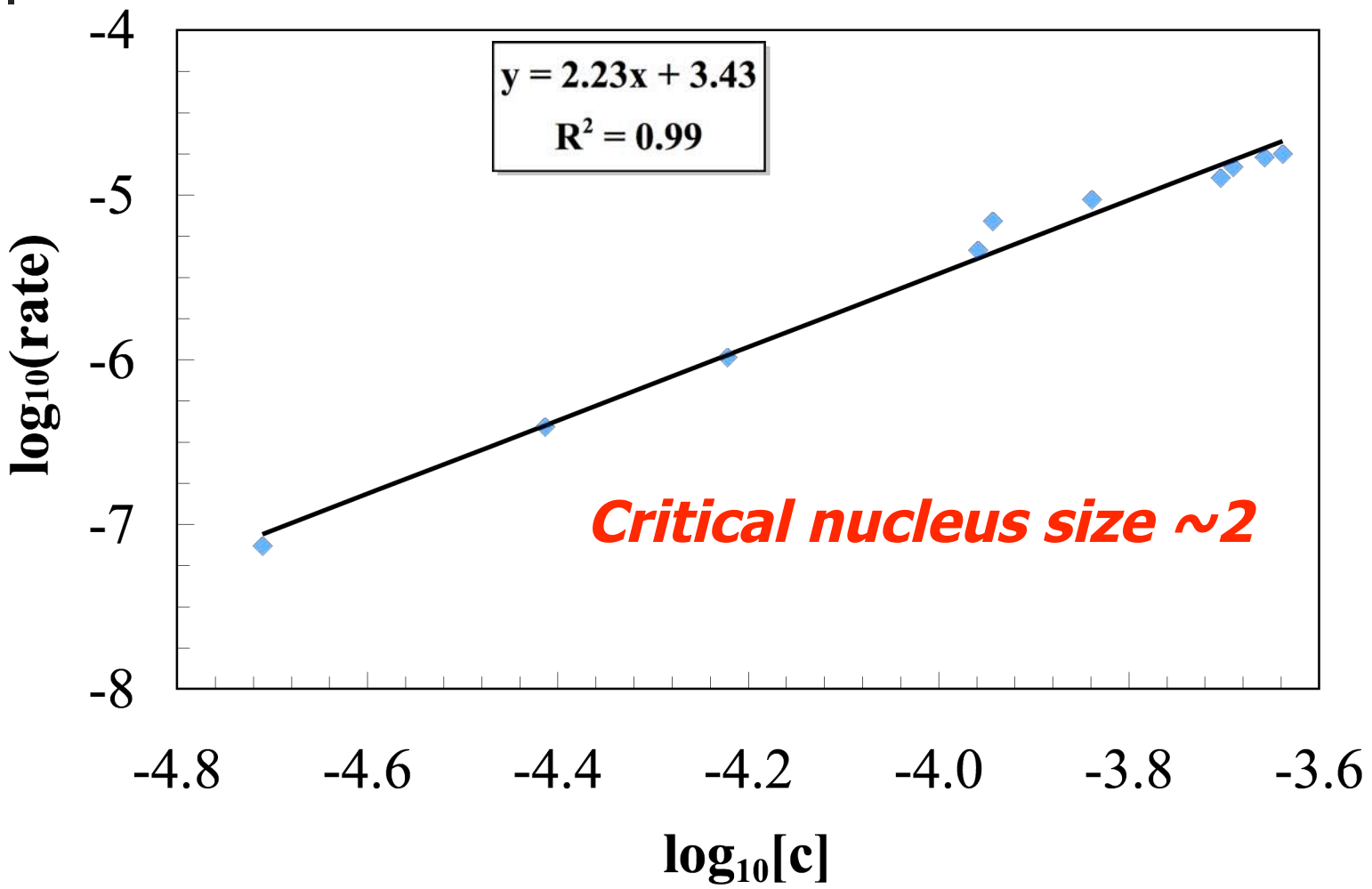
Zlotnick,A., J. Mol. Biol. (1994) 241,59-67,

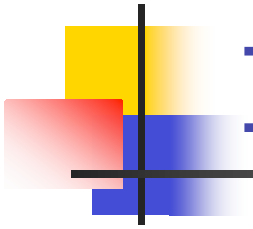
Prevelige,P.,Biophysical Journal, (1993) 64, 824-835

Slope of assembly rate



Critical nucleus is a dimer



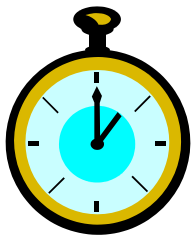


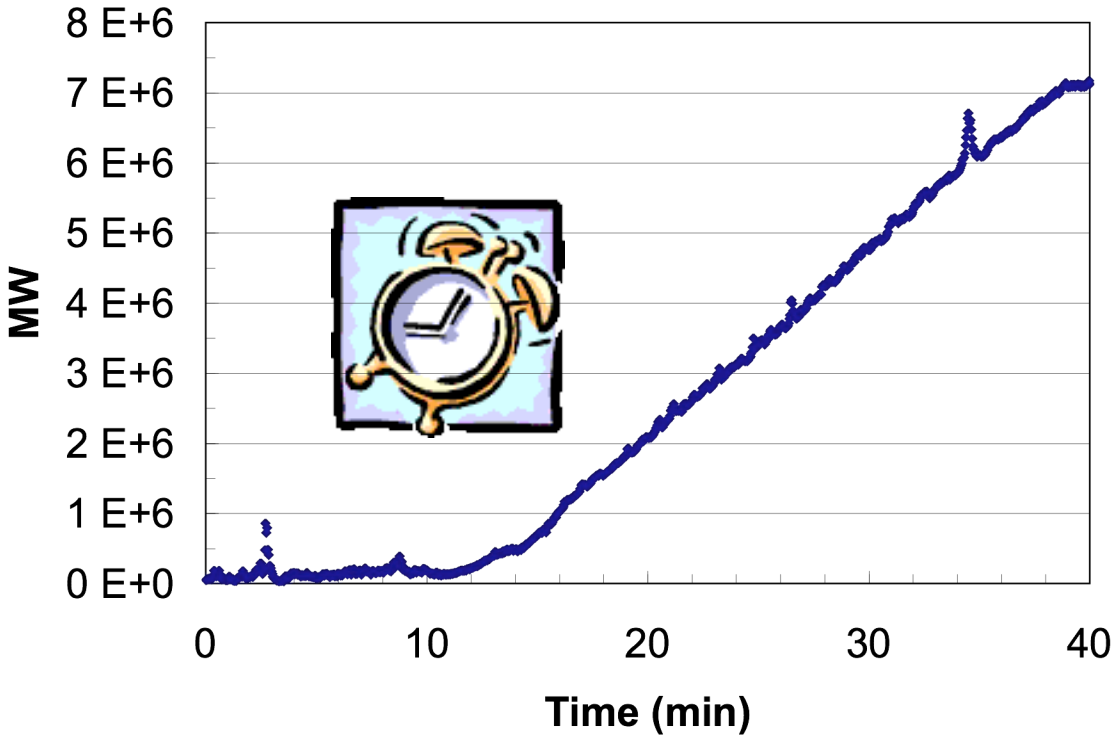
II. Why is lag time sharp?

- Perhaps transient nucleation is sharp
- Other kinetic mechanistic explanations?

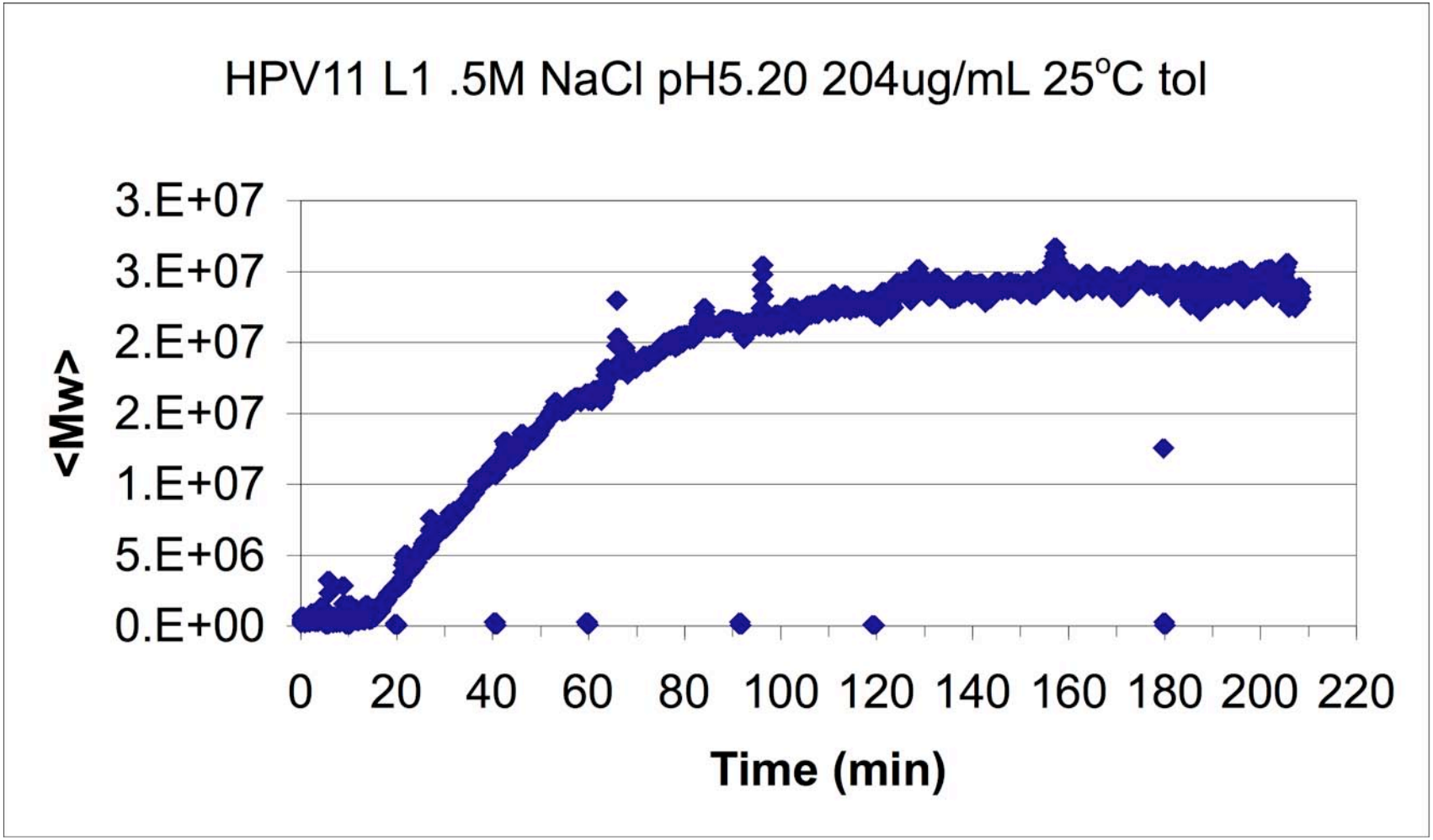
Question…

What type of mechanism shows a rapid increase after a reproducible lag?

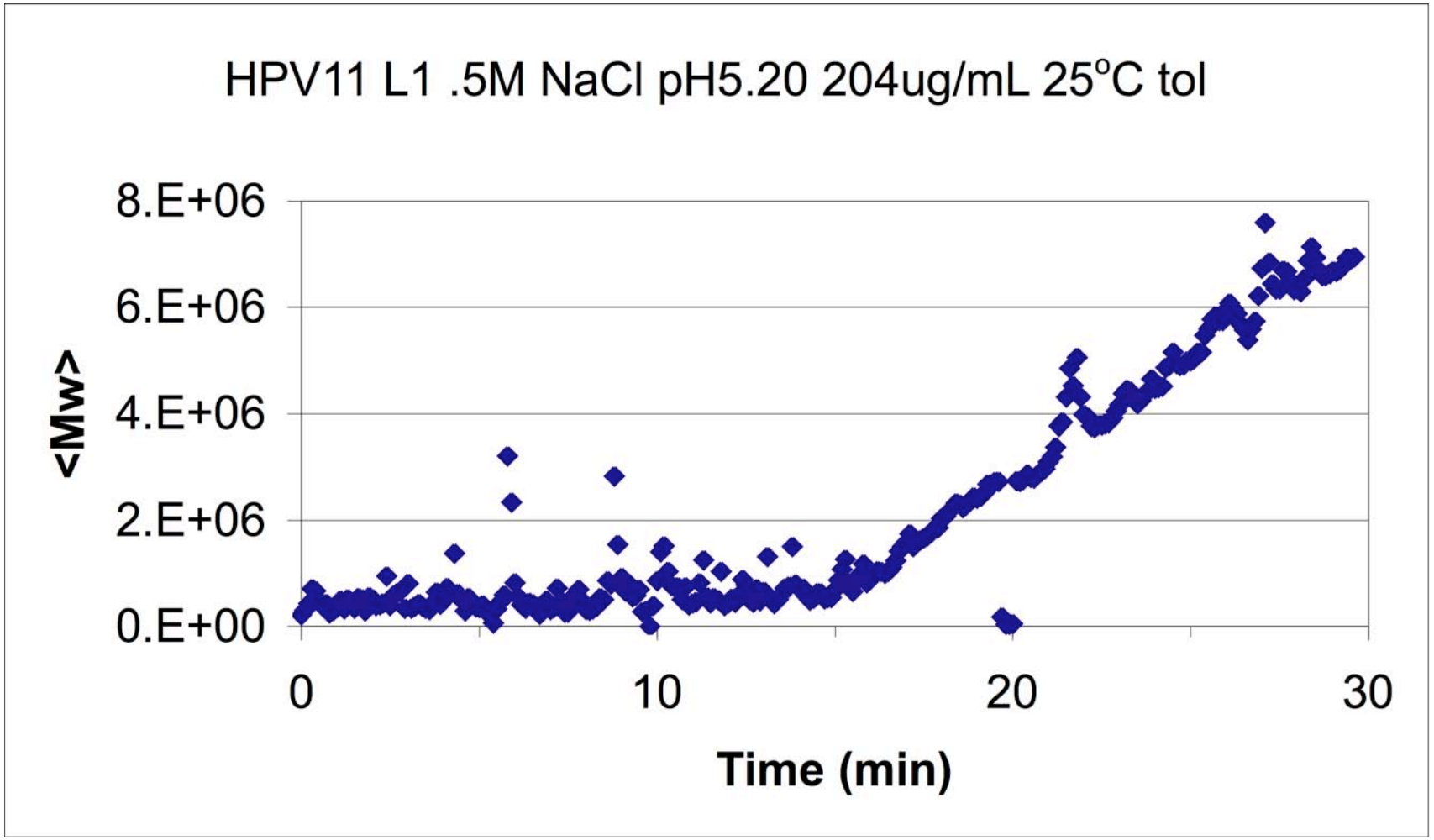




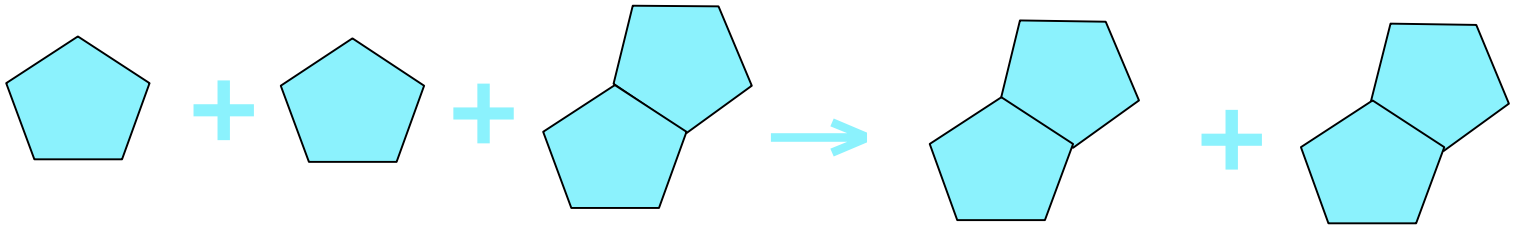
Lag time


HPV11 L1 .5M NaCl pH5.20 204ug/mL 25°C tol
<Mw>
3.E+07
3.E+07
2.E+07
2.E+07
1.E+07
5.E+06
0.E+00
0
20
40
60
80
100
120
140
160
180
200
220
Time (min)

Lag time close up



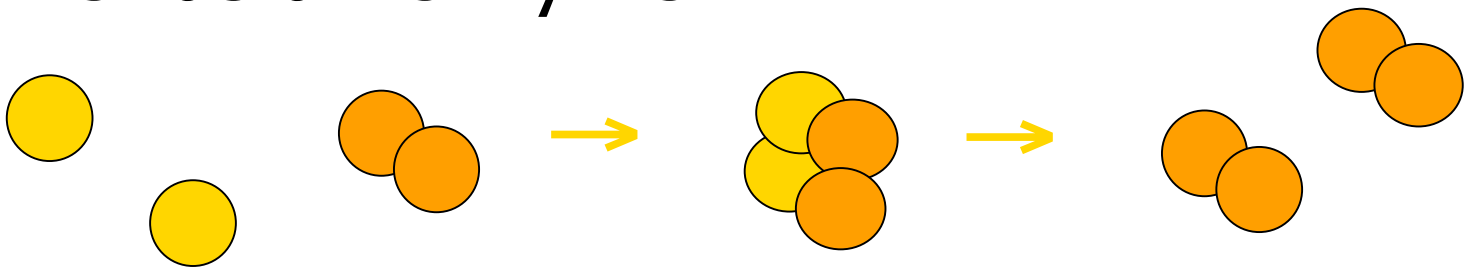
Autocatalysis



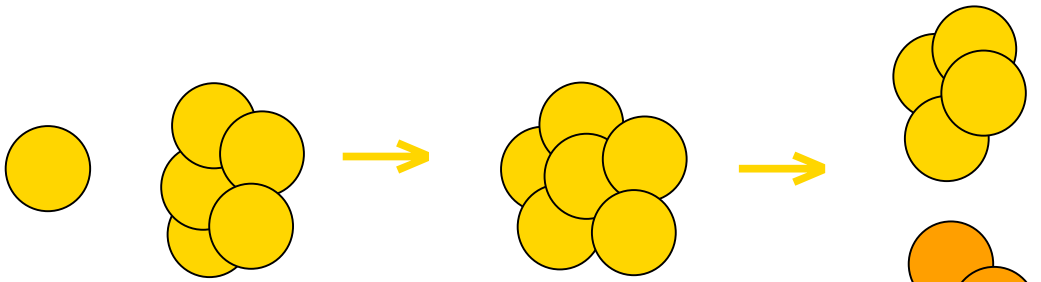
- Explains the sudden growth
- Supported by monomer binding

Variations on autocatalysis

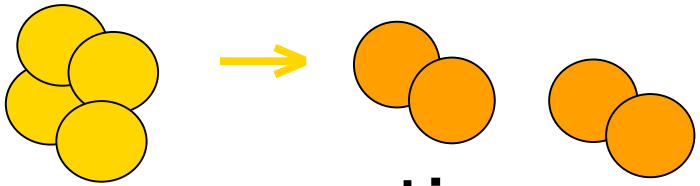
- Dimer as an enzyme



- Splintering



- Fragmentation



- Could help explain error correction

Possible mechanism

- Nucleation – low rate constant

$$C_1 + C_1 \xleftrightarrow{k_1, k_{-1}} C_2$$

- Autocatalysis – medium rate constant

$$2C_1 + C_2 \xleftrightarrow{k_a, k_{-a}} 2C_2$$

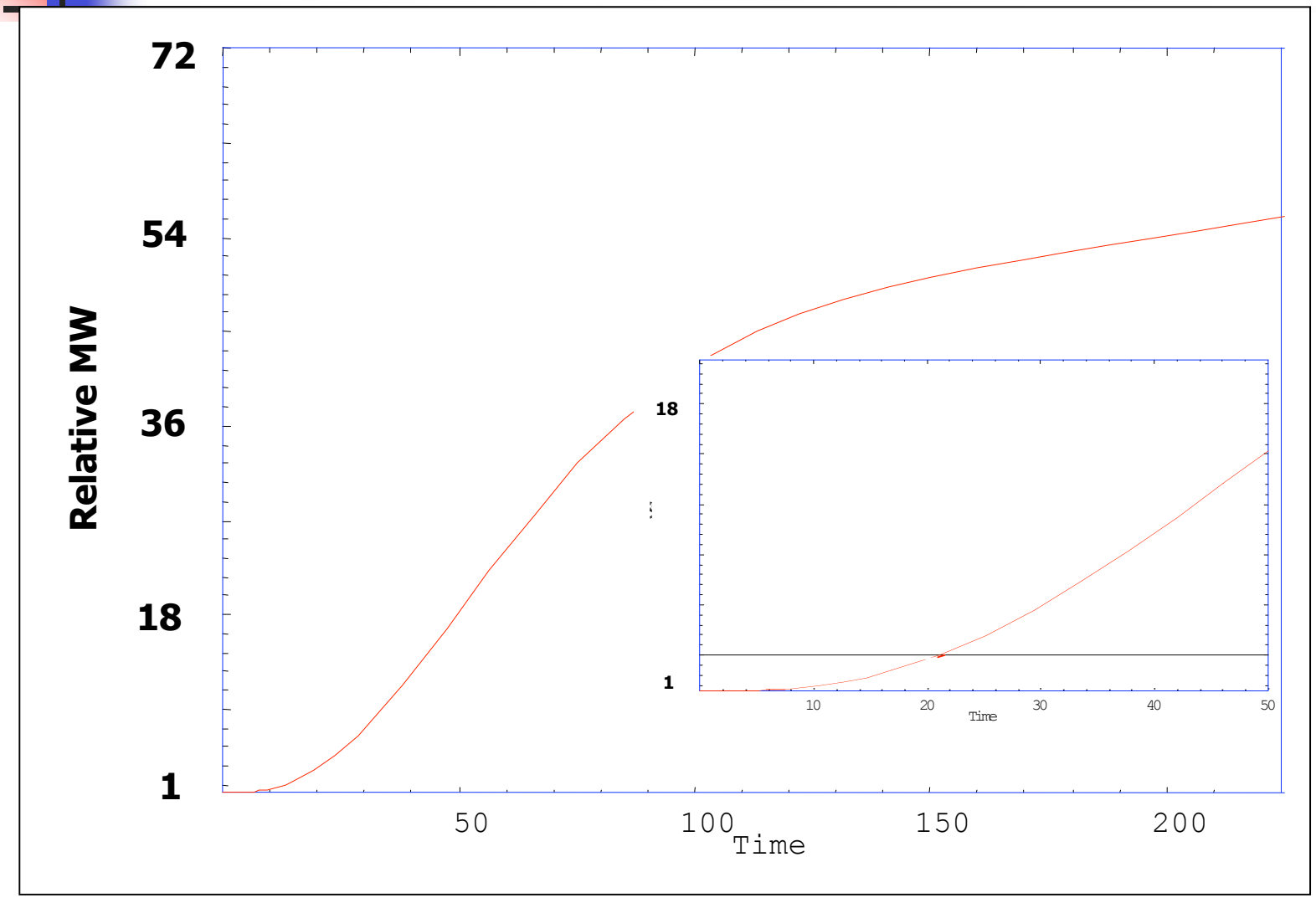
- Growth – high rate constant

$$C_2 + C_1 \xleftrightarrow{k_f, k_r} C_3$$

$$C_3 + C_1 \xleftrightarrow{k_f, k_r} C_4 \cdots$$

$$C_{71} + C_1 \xleftrightarrow{k_f, k_r} C_{72}$$

Model prediction of MW



Values:

C_o=.0002

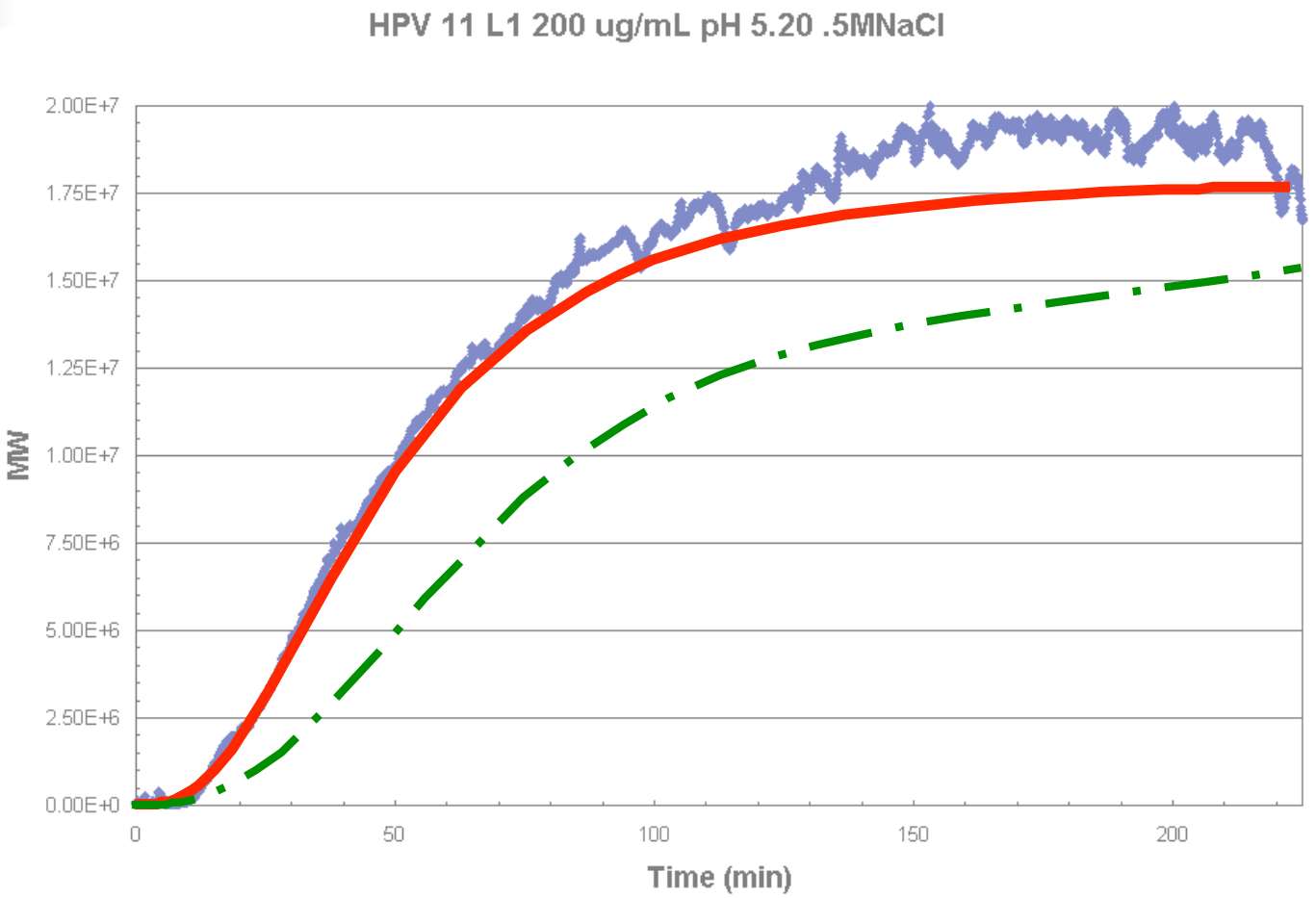
k_1=1

k_a=10^3

k_f=10^4

K_{eq}=10^5

Fit to experiment



Values:

$k_1=1$

$k_a=10^8$

$k_f=10^4$

$K_{eq}=10^5$

Values:

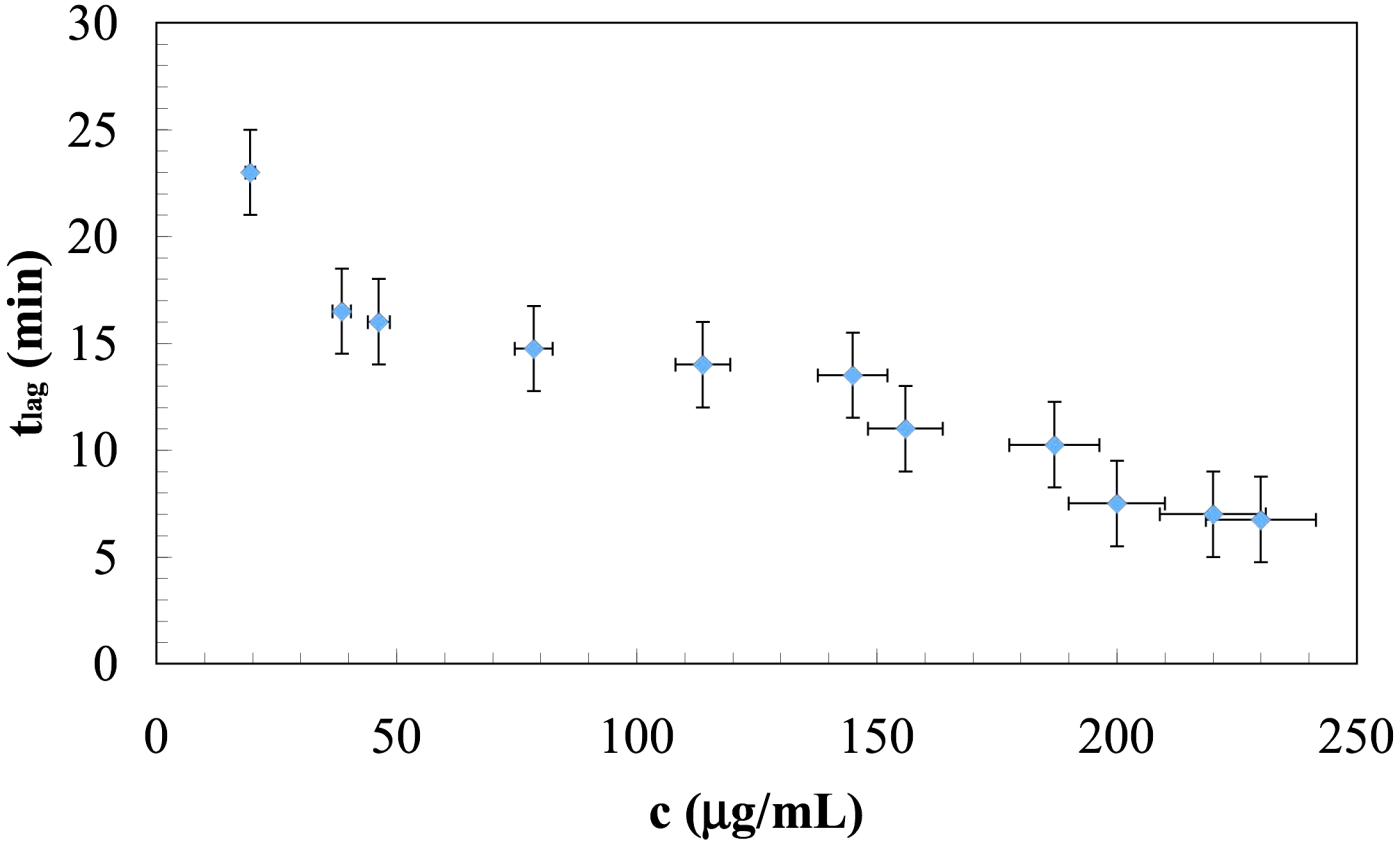
$k_1=1$

$k_a=10^3$

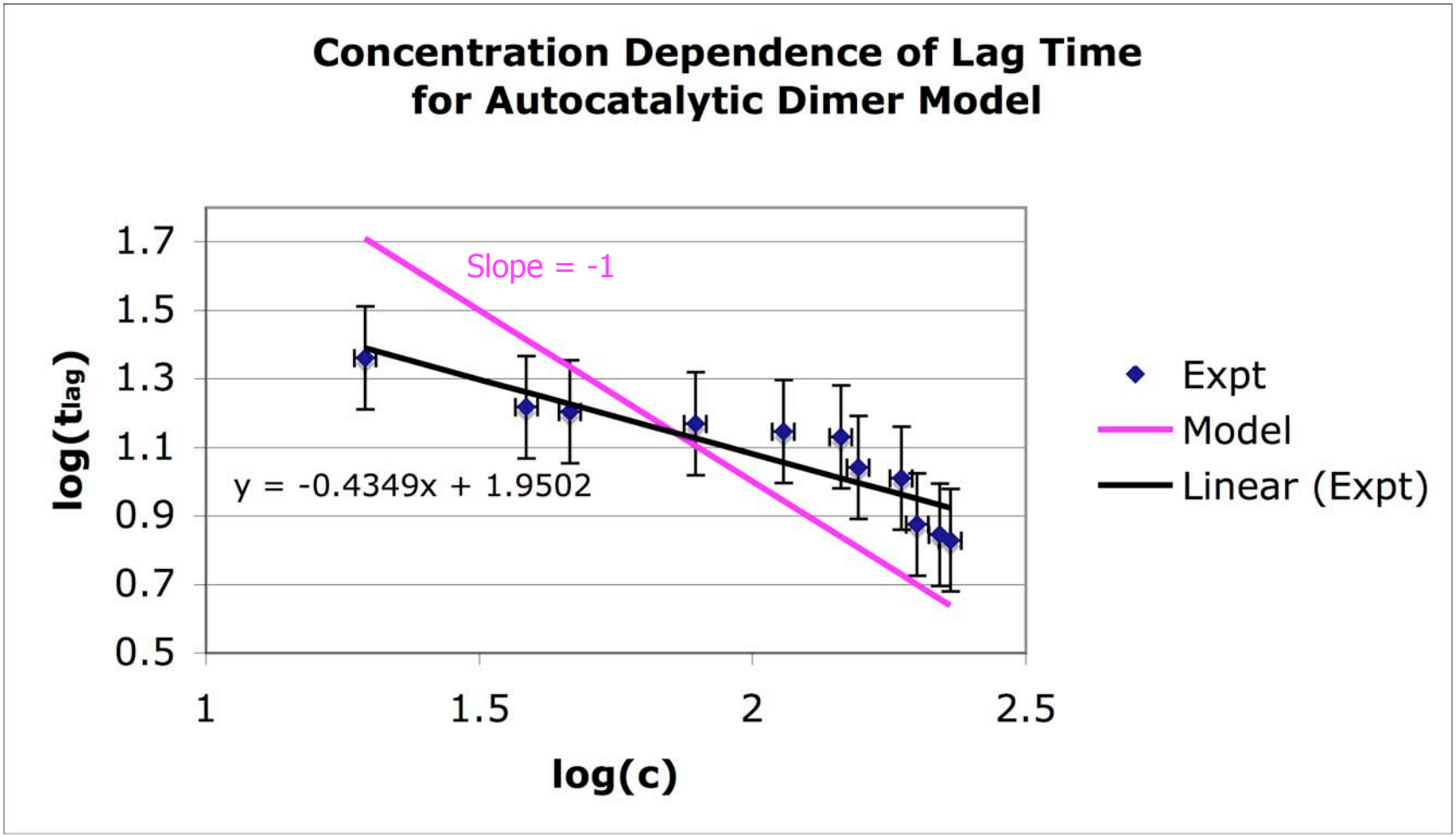
$k_f=10^4$

$K_{eq}=10^5$

Concentration dependence

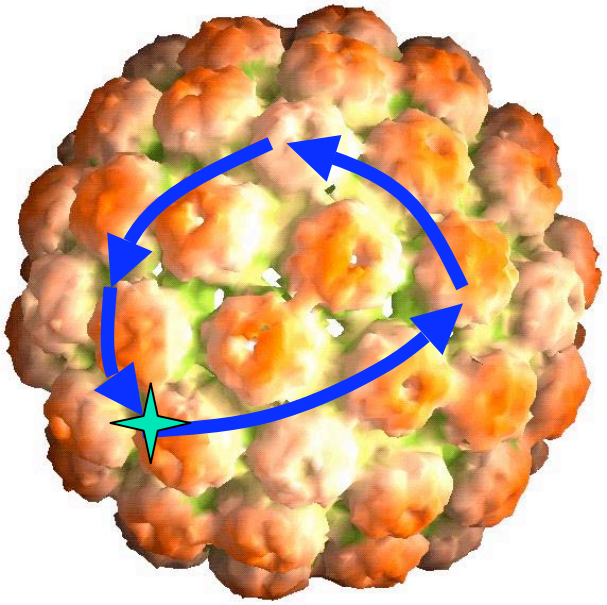


Comparison with model



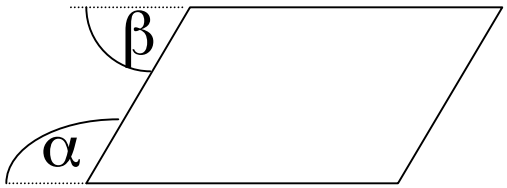
Places to visit next….

- This map may need more dimensions
- Error correction for quasiequivalent structures



Toy model for error correction

- 2D polygonal "capsid"
  - Different local binding conformations
  - Check for closing contact
  - Approximate check for overlap: exterior angles sum to < 360°

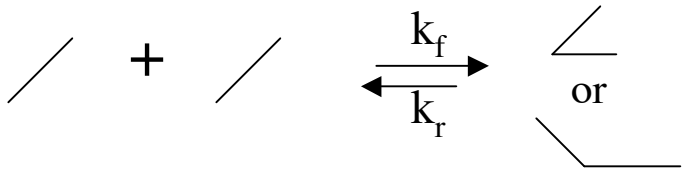


- Does the existence of "errors" qualitatively change assembly kinetics?
- If so, how are errors controlled?

Kinetic rate law model

Chemical Reactions ➡ Reaction Formulas ➡ Reaction Rates

Step #1



step #1

$M + M \xrightarrow{k} \alpha$

or

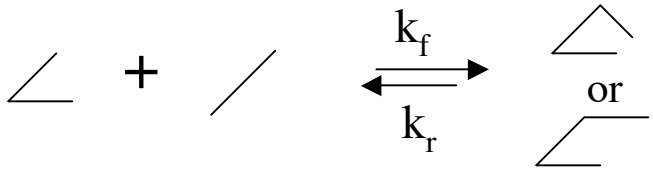
$M + M \xrightarrow{k} \beta$

$$\frac{d}{dt}[M] = -\left(4\,k\,M[t]^2 + k[\alpha][M] + k[\beta][M]\right)$$

$$\frac{d}{dt}[\alpha] = k[M]^2 - 2\,k[\alpha][M]$$

$$\frac{d}{dt}[\beta] = k[M]^2 - 2\,k[\beta][M]$$

Step #2a



step #2 a

$\alpha + M \xrightarrow{k} \alpha\,\alpha$

or

$\alpha + M \xrightarrow{k} \alpha\,\beta$

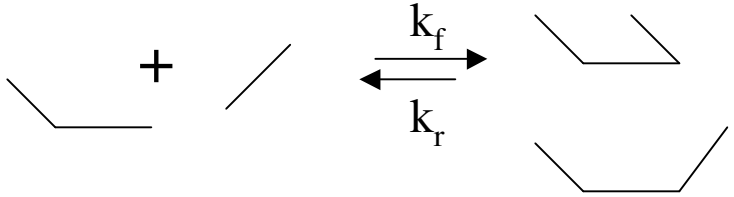
$$\frac{d}{dt}[\alpha\alpha] = -k[\alpha][M]$$

$$\frac{d}{dt}[\alpha\beta] = -k[\alpha][M]$$

$$\frac{d}{dt}[\beta\alpha] = -k[\beta\alpha][M]$$

$$\frac{d}{dt}[\beta\beta] = -k[\beta\beta][M]$$

Step #2b



step #2 b

$\beta + M \xrightarrow{k} \beta\,\alpha$

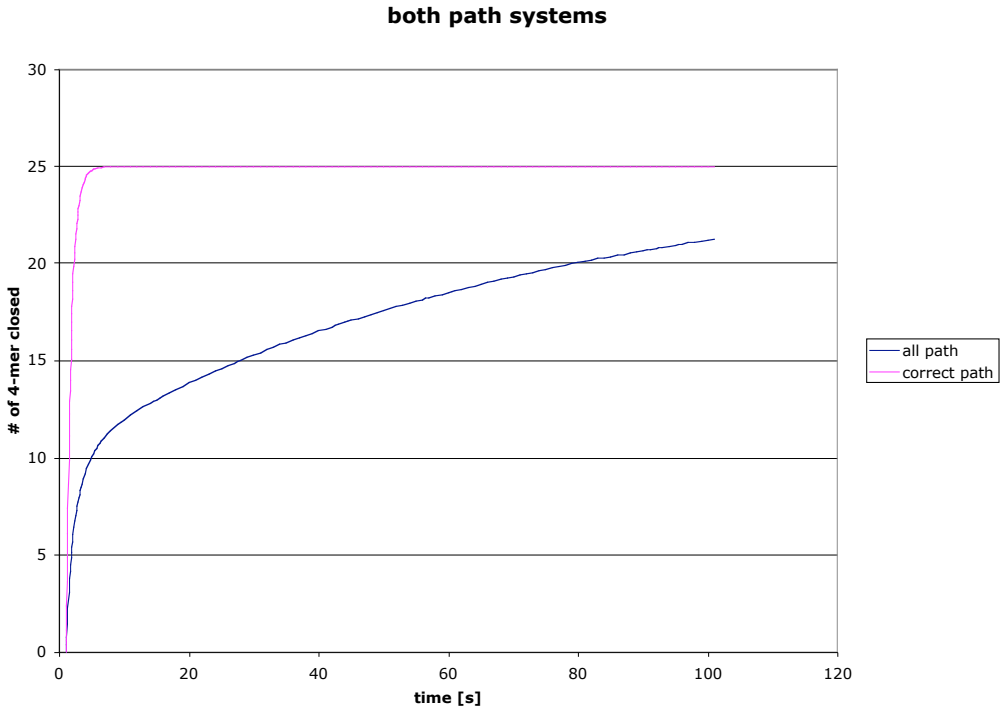
or

$\beta + M \xrightarrow{k} \beta\beta$

Evaluate multitude of reactions by stochastic MC

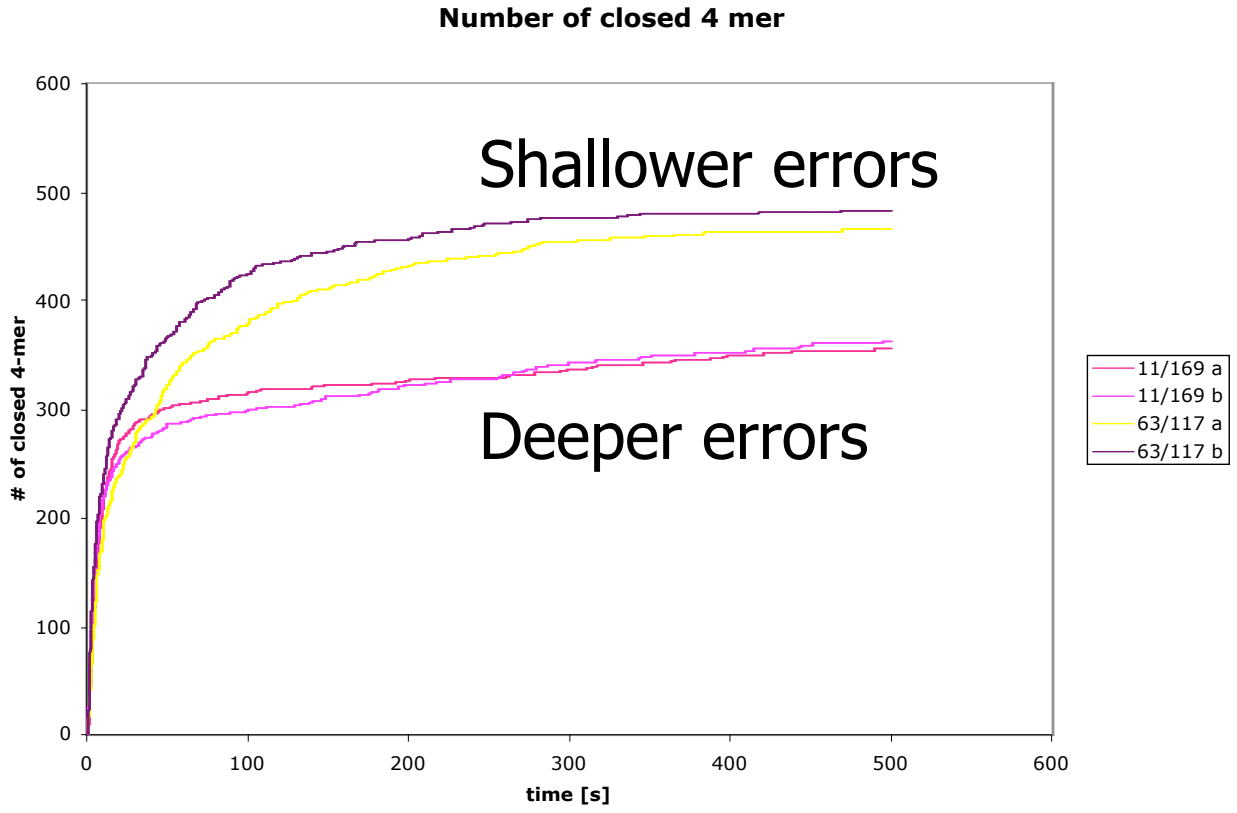
Possibility of errors produces 2 stage assembly

- Initial rapid assembly
- Slow conversion of incorrect structures
- Slow in comparison with Dominant Path mechanism
  - Using same rate constants

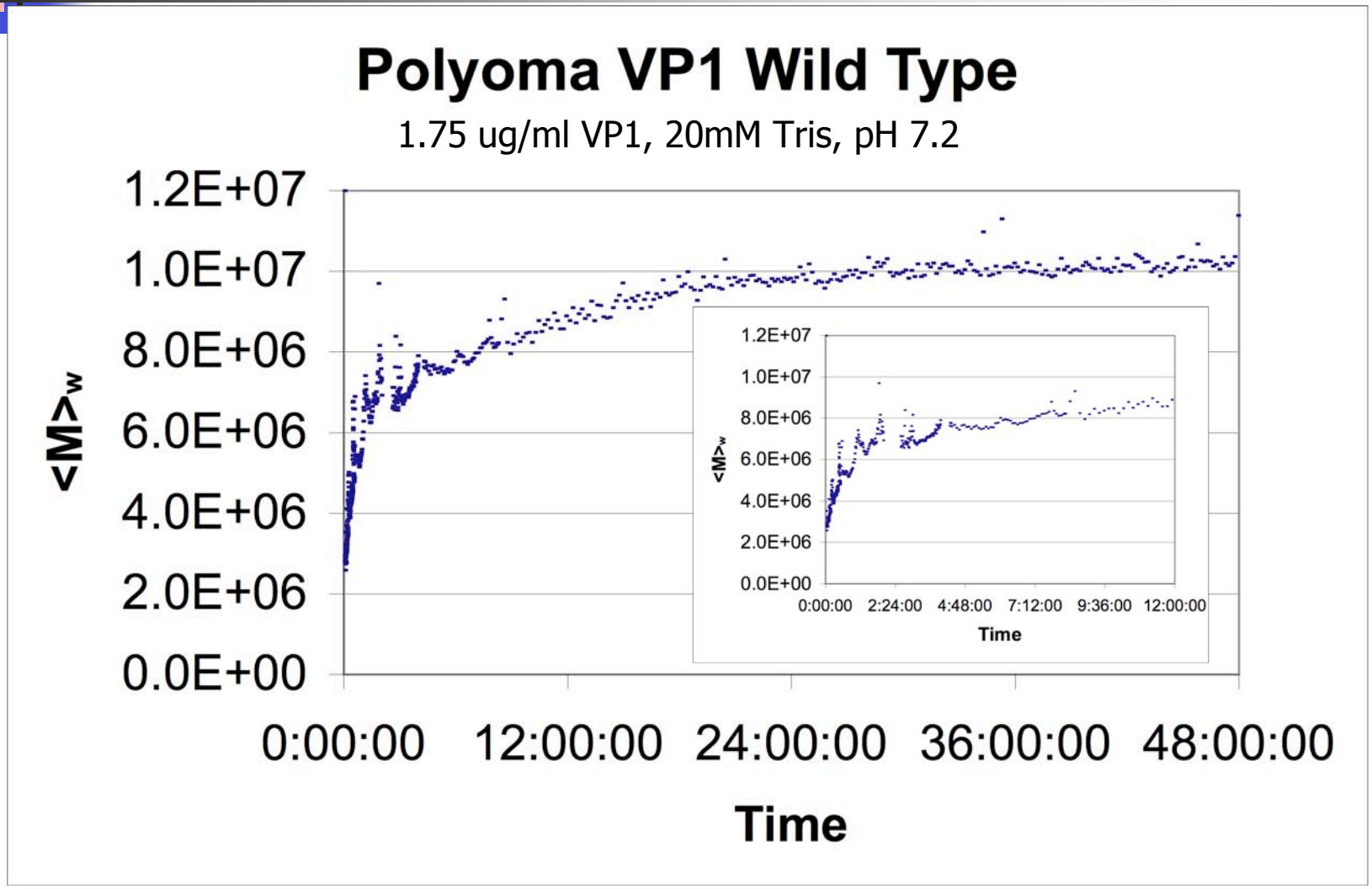


Depth of error matters

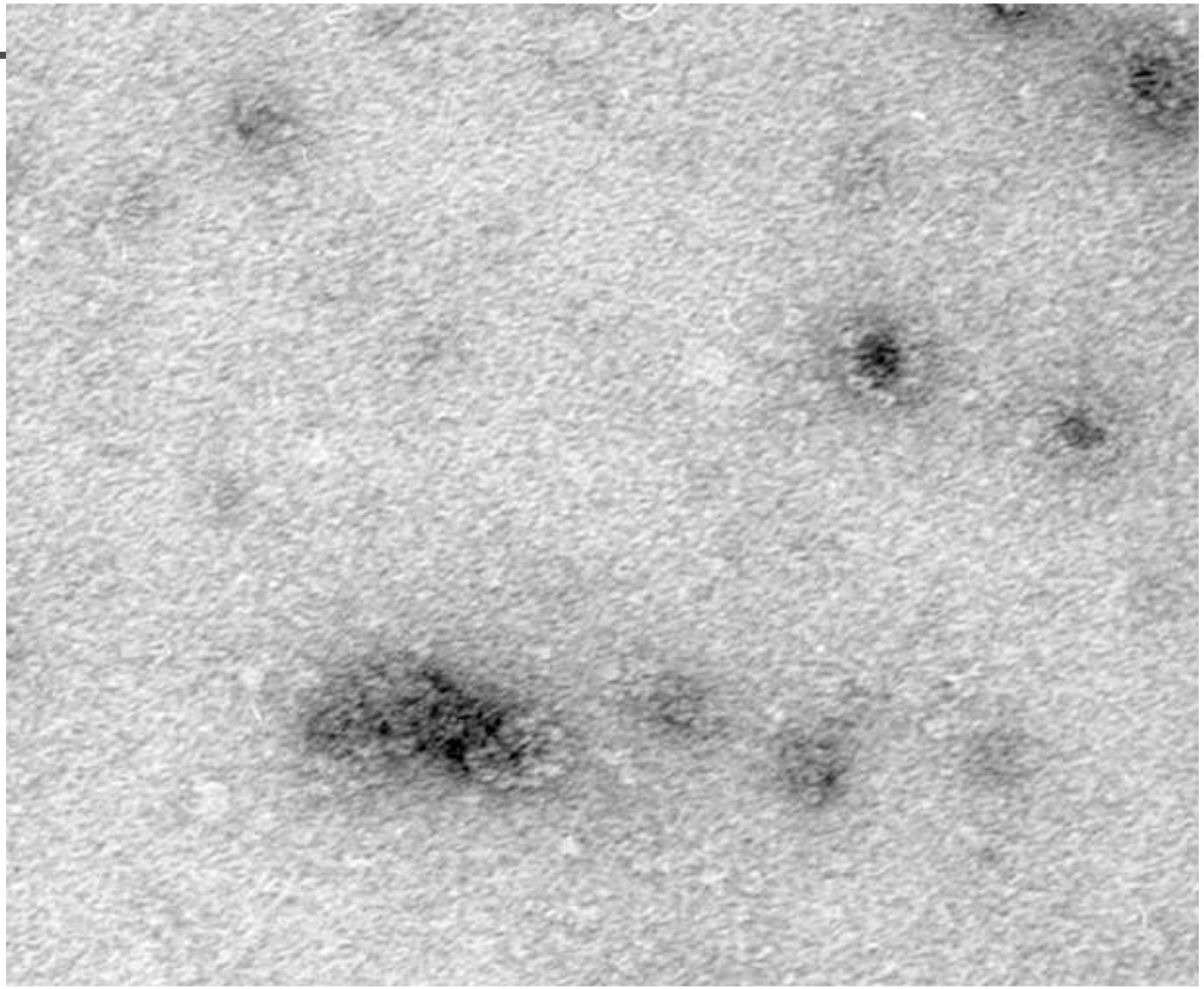
- Two rhombi compared:
  - $\alpha = 11^o$, $\beta = 169^o$
  - $\alpha = 63^o$, $\beta = 117^o$
- Two reverse rates compared:
  - a → k_r=1
  - b → k_r=2



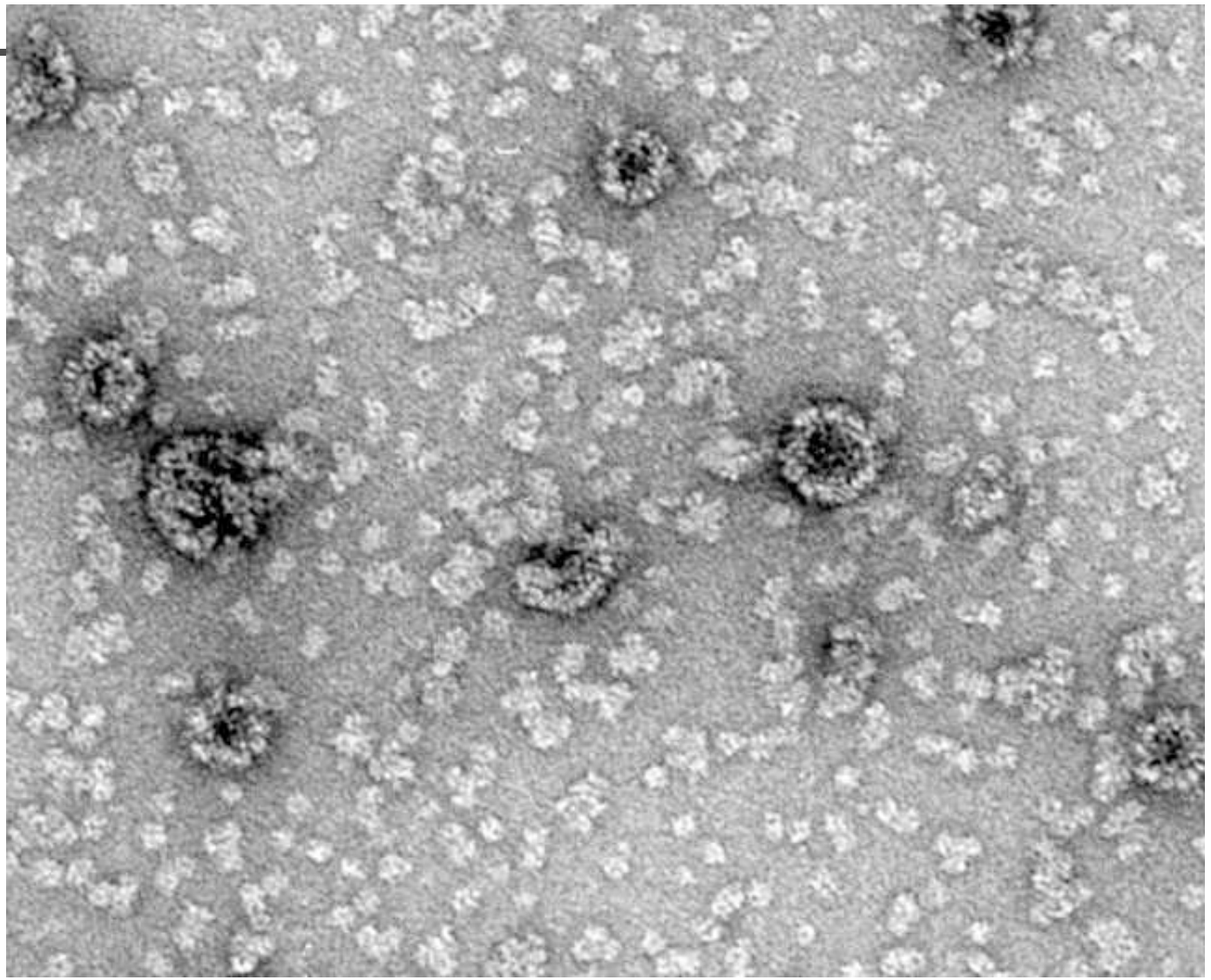
Polyoma assembly shows two-stage kinetics



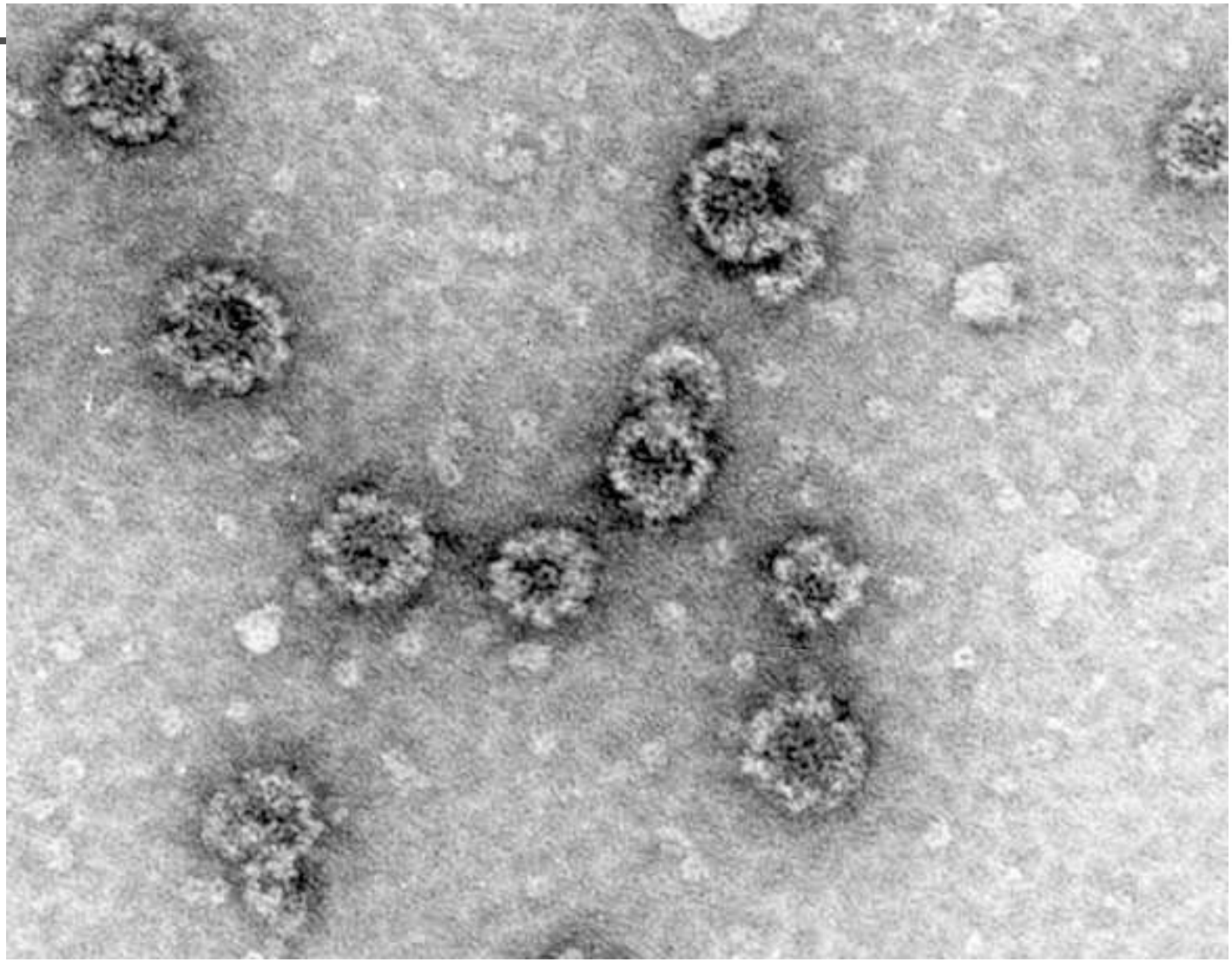
VP1 Assembly at 1M Salt (10 min)



VP1 Assembly at 1M Salt (1 hr)



VP1 Assembly at 1M Salt (1 day)



Conclusions…

- Experiments support a critical nucleus of a dimer for HPV assembly within nucleated dominant path mechanism
- Autocatalysis is a possible mechanism to explain the experimental lag time
- Model suggests, that once dimers are made, assembly to capsids proceed via an autocatalytic mechanism of those dimers

Conclusions

- Concentration dependence of lag time in the model does not fit experiment
- However, this dependence is a function of the specific autocatalysis mechanism

- Off-pathway assembly may be important
- Error correction for quasiequivalence
  - 2D Toy model shows error depth matters
  - In 3D, cooperative (e.g. strain) may limit error depth

Acknowledgements

- University of Colorado Health Sciences Center
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